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ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE THIRTY-FIFTH ANNUAL MEETING OF THE SOCIETY, COLUMBUS, OHIO, DECEMBER 4 TO 6, 1943

Blossom Drop of Wax Beans Reduced by Growth Substances. ALLEN, T. C., ELLSWORTH FISHER, AND A. J. RIKER. Bud, blossom, and pod drop have caused varying yield reductions in wax beans depending on conditions. These physiological diseases in northwestern Wisconsin have been associated with attacks by *Lygus* sp. insects and with long, hot summer days. To prevent abscission layer formation, growth substances were applied. Field dusts with naphthalene acetic acid at 140 p.p.m. increased yields 15 per cent in 1942. This material at 40 p.p.m. gave a 15 per cent field increase in 1943. Other compounds applied as dusts were less effective. In some trials yields were decreased because of chemical or mechanical injury. None of the sprays used was beneficial; some were harmful. Naphthalene acetic acid when too strong induced leaf symptoms suggesting virus infection. To study drop and its prevention by growth substances in greenhouse trials, abscission layers were stimulated in both bean petioles and pedicels by a day's treatment with illuminating gas. Effective treatments started with about one part of gas in 135 parts of air at about 29° C. The growth substances tested were comparable in effectiveness in greenhouse and field, suggesting that perhaps other abscission diseases may be studied advantageously with illuminating gas along with growth substances.

Action of Clavacin on Certain Phytopathogenic Bacteria. ANDERSON, H. W. A mycogenous bactericide, named *clavacin* by Waksman, was tested against a number of phytopathogenic bacteria. A strain of *Aspergillus clavatus*, furnished by Waksman, was used in the production of the test clavacin. The sterile, unconcentrated filtrate contained approximately 4 Oxford units per ml. Twenty bacterial species were used, representing 7 species of *Xanthomonas*, 5 of *Pseudomonas*, 5 of *Corynebacterium*, 2 of *Agrobacterium*, and 1 *Bacterium* (*B. stewarti*). A standard strain of *Staphylococcus aureus* was included for comparison. Results were expressed in per cent of filtrate necessary to: (1) kill bacteria (lethal dose), (2) inhibit growth (bacteristatic action) in dextrose broth. On plates the potency was determined by measuring the cleared rings. Lethal dose for various species varied from 2 to 6 per cent. Four species were killed at 2 per cent or less, 9 at 4, and 2 species at 6. Five were not killed at 6 per cent, but bacteristatic action was observed in these at 4 per cent. Bacteristatic action ranged from 1 to 4 per cent, but 10 of the 20 species were inhibited at 1 per cent or less and seven between 2 and 3 per cent. Fifteen of the 20 organisms were *Gram negative*. There seemed to be no relation between lethal or bacteristatic doses and reaction to the Gram stain. *Bacterium stewarti* and *Xanthomonas pruni* were most sensitive. *Staphylococcus aureus* reacted in much the same way as these 2 phytopathogenic, Gram negative bacteria in both cell and dilution methods of testing. Penicillin was entirely ineffective against *Xanthomonas pruni*.

Pathogenicity for Tobacco of Some Bacterial Isolates Antigenically Similar to Bacterium tabacum. BEACH, W. S., AND PAUL SACCO. Comparative pathogenicity for tobacco was determined for 62 cultures of bacteria selected from a series of 603, isolated by Reid *et al.* (Penn. Exp. Stat. Bull. 422, 1942) from healthy clover and tobacco and tobacco soils. They reported these 603 isolates among others to have similar agglutination reaction with *Bacterium tabacum* used as the antigen. Following heavy atomization of succulent leaves of Pennsylvania Broadleaf tobacco, 36 isolates, including some from each stated source, produced typical wildfire spots, while 26 isolates gave no apparent infection. A few of the latter, however, showed slight invasion when leaf tissues were water-soaked by a forced spray. These few possibly represent strains of *Bact. angulatum* of low virulence. Isolates showing weak wildfire infection acquired greatly increased virulence by 7 daily passages through either of two liquid media, one composed of mineral salts and 5 per cent asparagin, the other of unheated tobacco juice and 2.5 per cent asparagin. The increased virulence appeared due to greater abundance of the halo-producing toxin. The initially non-infectious isolates acquired no appreciable degree of pathogenicity by passage through these media. This non-infectious character of a large proportion of the isolates is further evidence that similar agglutination may not signify similar pathogenicity. The isolation of *Bact. tabacum* from soil tends to substantiate field observations that this pathogen overwinters in Pennsylvania soil.

Scab of English Ivy Caused by Sphaceloma. BITANCOURT, A. A., AND ANNA E. JENKINS. A leaf scab of English ivy (*Hedera helix* L.) has been discovered in southern California, whence specimens from the vicinity of Los Angeles, Mar Vista, and Upland, collected in 1940–1943, have been communicated by H. S. Fawcett. In March of the

current year the disease was found in São Paulo, State of São Paulo, Brazil. The spots are few to numerous, scattered or confluent, occasionally present on veins, usually circular, but sometimes irregular, on the lower leaf surface 1 to 5 mm. in diam., more or less abruptly raised, sometimes depressed at the center, brown, often Verona brown to warm sepia, sometimes ecru drab; above, less prominent and smaller, 1 to 2 mm. in diam., with abruptly raised margin, sometimes purple-bordered to the extent of the spot above. This disease evidently is caused by *Sphaceloma* which is present on the spots and which has been isolated from the specimens from Mar Vista and Sao Paulo.

Two New Viruses Transmitted by Agallian Leaf Hoppers. BLACK, L. M. The discovery that of the two agallian leaf hoppers, *Aceratagallia sanguinolenta* and *Agallia constricta*, only the former transmits the New York variety of potato yellow-dwarf virus and only the latter transmits the New Jersey variety suggested that other agallian leaf hoppers might be carrying other yellow-dwarf viruses in nature. To test the hypothesis, *Agalliopsis novella* was selected for investigation. Collections were made with the collaboration of P. W. Oman. When this species was tested on crimson clover, two new viruses were revealed. One of these is transmitted only by *Agalliopsis novella* and the other by this species and also by *Agallia constricta*. The relationships of the new viruses are uncertain.

On the Survival of Corynebacterium sepedonicum under Various Storage Conditions. BONDE, REINER, AND S. F. SNIESZKO. Samples of two potato soils from Aroostook County, Maine were sterilized in bottles and inoculated with pure cultures of *Corynebacterium sepedonicum* or crude cultures in form of crushed ring-rot-infected potato tubers. Bottled inoculated soils were buried in November in the ground in Aroostook Farm and left until the end of May. In spite of precautions, sterilized soils in many bottles became infected with various soil microorganisms. From the soils inoculated with pure cultures, *C. sepedonicum* was recovered in 7 out of 18 cases in Washburn loam and in only one out of 18 in Caribou loam. Potato seed pieces infected through placement in some of the soils inoculated with pure cultures, produced ring rot-infected plants. In none of the 36 soil samples inoculated with crushed infected potato tubers could the presence of virulent or viable *C. sepedonicum* be demonstrated. Also, small pieces of cotton and jute potato bags were sterilized and inoculated with pure and crude cultures of *C. sepedonicum* and stored under various conditions since November. Only from samples inoculated with pure cultures were viable and virulent cultures of *C. sepedonicum* recovered 4 to 9 months later.

The Influence of Guttation Fluid on Pesticides. CURTIS, LAWRENCE C. Guttation water, collected from different vegetable plants, was shown to contain varying amounts of total solids about half of which was of an organic nature. The guttation fluid, when sprayed on glass slides previously coated with Bordeaux mixture and yellow copper oxide, increased to a marked degree the per cent of inhibition of spore germination of *Macrosporium sarcinaeforme*. Various concentrations of different salts gave similar results. Experimental evidence supports an hypothesis to explain how the copper in Bordeaux mixture is rendered soluble by the guttation water and how this soluble copper enters the leaf to cause injury to susceptible plants or to prevent tipburn on potato plants.

Deleterious Effects of Guttated Fluids on Foliage. CURTIS, LAWRENCE C. Both in the field and greenhouse it was observed that 3 things may happen to the guttation drop on plants: (1) it may roll off, (2) it may evaporate, and (3), as most frequently happens on undisturbed plants, it may be sucked back into the leaf. When neutral red crystals were placed in the guttation drop of maize and squash plants, the drop was immediately sucked back into the leaf, and the vascular system of this leaf was stained for a distance of 1 to 2 inches.

An hypothesis is advanced to explain tip burn. When the guttation water evaporates on the tips of the leaves, it leaves various salt deposits that may increase due to a continuous guttation process over a 1-, 2-, or even 3-day period. These deposits may affect the leaf in 2 ways: because of their high salt concentration they may remain and damage the outside of the leaf in the same way that fertilizer does when it is put on leaf blades, or they may go into solution in subsequent guttation water and be sucked back into the leaf where the hypertonic solution kills the cells. New products or changes in the guttation fluid, which are toxic to the internal cells when the fluid is sucked back, are produced either by bacteria, molds, or enzymes.

Soil Treatments with Sodium Nitrite for Controlling Damping-off and Root Knot. ELLIS, D. E. Results of greenhouse and plant bed tests in 1942 and 1943 indicate that sodium nitrite is effective in controlling Rhizoctonia damping-off of lettuce. Four and 8 ounces per square yard applied to artificially contaminated plant bed soils 4 weeks before seeding reduced post-emergence damping-off by 71 and 95 per cent, respectively. The

higher rate slightly reduced stands but gave better control than chloropicrin, formaldehyde, or urea. At the same rates, under field conditions, sodium nitrite, applied to soils naturally infested with *Heterodera marioni* 7 weeks prior to planting, reduced root knot infection of bean, okra, squash, and tomato. At the higher rate control was equal or superior to that afforded by chloropicrin or urea. Root knot index readings ranged up to 3.2 for the lower and 1.0 for the higher rate as compared with 56.0 to 71.5 for the controls. Comparable figures on the basis of percentage of plants affected were 16, 3, and 76 to 100, respectively. Counts showed that the treatments reduced stand only in the case of beans. Observations indicate that stand reductions may be largely overcome by earlier application.

Sodium Nitrite for Root-knot Control. FELDMAN, A. W., AND LUTHER SHAW. During the past 2 years a number of chemicals have been employed as soil treatments in an effort to find a control for the root-knot nematode (*Heterodera marioni*). Of the chemicals applied, sodium nitrite proved most effective. Under greenhouse conditions, using tomatoes as the host, concentrations as low as 6 oz. per sq. yd. have given 93 per cent control, and 12 oz., 100 per cent control. Plant response to this treatment was quite noticeable in comparison with other treatments and checks. Not only was rapid growth produced, but greener foliage, good fruit set, and well-developed roots resulted. A method of standardizing chemical applications to various soil types was employed. The base exchange capacity and replaceable hydrogen were determined, and sodium nitrite was added in equivalents of 50, 100, 150, and 200 per cent of the replaceable hydrogen. Greenhouse and laboratory tests showed a rapid transition of the NO_2^- to NO_3^-, temperature and moisture being important factors. Good growth was obtained when plantings were made as early as 1 week following application of the chemical. Microflora analyses of the soils under field conditions indicate that the chemical is highly toxic to soil organisms.

Electrophoretic Studies on Sap from Plants Infected with Tobacco-mosaic Virus. FRAMPTON, VERNON L., AND W. N. TAKAHASHI.

Expressed Sap of Tomato Plants in Relation to Wilt Resistance. GOTTLIEB, DAVID. Sap expressed from stems of 3 varieties of tomato retarded the growth of *Fusarium bulbigenum* var. *lycopersici* in proportion to the wilt resistance of the variety. The average yield of mycelial mats in sterile expressed sap were 0.145, 0.110, and 0.065 g., for Bonny Best, Marglobe, and Pan American, respectively. Similar results were observed when the saps were mixed with equal parts of 3 per cent potato dextrose agar and the linear growth measured. After 10 days the average size of the colonies on media from the above-mentioned varieties were 81, 74, and 65 mm., respectively. The retarding material in the sap was stable at 100° C. for 2 hours, and was absorbed by activated charcoal at room temperature. It can be distilled at 95° C. under reduced pressure and the condensed distillate retains its inhibiting properties.

Seed-borne Diseases of Cereals in Manitoba—A Survey, 1937–1942. GREANEY, F. J., AND J. E. MACHACEK. During the 6-year period 1937–1942, a large number of samples of wheat, oats, barley, and rye seed were collected each year in Manitoba and examined for the presence of parasites causing seed-borne diseases. Plating tests of the seed showed that, among the numerous organisms isolated, species of *Helminthosporium* (*H. sativum* on wheat, barley, and rye; *H. avenae* on oats; and *H. teres* on barley) were the most important pathogens. Several species of *Fusarium* were commonly isolated, particularly from oat seed, but they were not harmful. Soil-bed tests showed a high positive correlation between the percentage of seeds infected with *H. sativum* and *H. avenae* and the amount of disease in the subsequent seedlings. In wheat, but not in barley, seed infection with *H. sativum* was associated with low germination. Of the wheat samples tested 96.4 per cent were virtually free from surface-borne smut; whereas over 80 per cent of the oat and barley samples carried a smut spore load sufficiently high to make seed treatment necessary. Each year, nearly half (46 per cent) of the Manitoba seed stocks of wheat, and more than 80 per cent of those of oats and barley, required seed treatment for disease control.

The Effect of Plant-decomposition Products on Root Diseases. GRIES, GEORGE A. The variable effect of different crop residues on strawberry root rot is known. In the experiments herein reported crop plants were decomposed in 2-gal. crocks. The extracts produced were sterilized by filtration and their toxicity was tested on strawberry and tobacco plants grown in water culture. The sterile extract of red clover, decomposed by its natural microflora, was decidedly toxic to the test plants, whereas the extracts of soy beans similarly produced were innocuous. When these plants were sterilized and decomposed by the microflora peculiar to the other host, the order of toxicity was reversed. This indicated that the evolution of toxic decomposition products is largely a function of the

organisms involved. Variations in the toxicity of extracts from various collections of timothy showed considerable difference in the type of microflora that may be associated with a given crop. The effect of certain crop rotations on the incidence of parasitic diseases was indicated by the toxicity of many of the sterile extracts to pathogenic fungi in culture.

Juglone (5-hydroxy-1, 4-naphthoquinone)—A Promising Fungicide. GRIES, GEO. A. Juglone (5-hydroxy-1, 4-naphthoquinone), the oxidized form of hydrojuglone found in members of the genus *Juglans*, is toxic to certain higher plants growing in the proximity of walnut trees. In standardized laboratory tests for fungicidal activity on a series of naphthoquinones and related compounds, juglone most nearly approached the toxicity of the copper in Bordeaux mixture. The L. D. 50 values of freshly extracted or synthesized juglone using *Sclerotinia fructicola* and *Macrosporium sarcinaeforme* as test organisms were 0.19 and 0.27 micrograms per sq. cm., respectively. These data were based on a spore load of 50,000 spores per cu. cm. The L. D. 50 values increased slightly over a 6-mo. period under laboratory conditions, suggesting a tendency for juglone to decompose to by-products of a lower order of toxicity. Greenhouse tests proved juglone to be ineffectual as a contact or stomach insecticide. Although the toxicity of juglone to the root systems of higher plants was confirmed, no foliage injury was evident when it was sprayed on cutinized surfaces. Tests in which juglone was used as a seed protectant indicated that it was extremely deleterious to the non-cutinized surfaces of germinating seeds. In preliminary field experiments, promising results were obtained with juglone for the control of black spot of roses.

Life History of Hypoxylon pruinatum in Relation to Pathogenicity on Aspen. GRUENHAGEN, R. H. Aspen, in the Lake States, is used extensively for boxwood, pulp wood, and excelsior, and is a potential source of raw cellulose for explosives and plastics. Stands examined had 10 to 60 per cent of the trees infected with *Hypoxylon pruinatum*, which was found to enter only through bark wounds. The best results from inoculation were secured when the fungus was introduced through a wound near the center of a bruise. An active infection girdled a 4-inch tree in 2 to 3 years and spread vertically 3 to 6 feet. The fungus was seen only in microscopic sections of the cortex, cambium, and outer wood. Conidia were borne on conidiophores at the surface of the canker, as well as on raised mycelial pillar-like structures. Conidia were abundant during spring and early summer. Ascospores were forcibly discharged from April through September out of perithecia in stromata raised above the canker surface. Both conidia and ascospores were wind-borne. Warm, rainy weather, followed by periods of high humidity favored spread of the disease. The organism overwintered both as ascospores and as mycelium. The organism on 2 per cent malt agar grew best at 28° C. and at pH 5.0 to 6.0.

Effect of Seed Treatment on the Control of Oat Smut. HANSING, E.D. Quantities of Kanota oat seed inoculated artificially by dusting with chlamydospores and by partial evacuation with a suspension of chlamydospores, and naturally infected seed from the previous season were treated with different fungicides. Each treatment was replicated and the data analyzed by analysis of variance. Seed inoculated by dusting and then treated produced average smutted panicles as follows: checks 38.7%, seed treated with volatile fungicides (New Improved Ceresan, DuBay 1452-C (Ethyl mercury p-toluene sulfonanilide), and formaldehyde) 0.6%, and seed treated with "nonvolatile" fungicides (Arasan, Corona Coppercarb, micronized free-flowing sulphur, micronized wettable sulphur, Spergon, Yellow Cuprocide, and yellow mercuric oxide) 2.7%. All fungicides gave significant control of smut. Seed inoculated by partial evacuation produced average smutted panicles as follows: checks 60.9%, seed treated with volatile fungicides 8.0% and seed treated with nonvolatile fungicides, 63.4%. Thus, only volatile fungicides gave a significant reduction in percentage of smut. Naturally infected seed produced smutted panicles as follows: checks 34.2%, seed treated with volatile fungicides 0.4%, and seed treated with nonvolatile fungicides 5.0%. Although all of the seed treatments reduced the percentage of smut significantly, satisfactory control was obtained only by the use of the volatile fungicides (volatile vs. nonvolatile, odds $> 999:1$).

Studies on the "Black Point" Disease of Wheat in the United States. HANSON, E. W., AND J. J. CHRISTENSEN. Prevalence, severity, causes, distribution, symptoms, and effects of the seed discoloration commonly known as "black point" were studied from 1935 to 1942 on 5 to 25 varieties of bread and durum wheats obtained from 13 States. The amount of seed discoloration, the fungus flora concerned, and all other factors studied varied greatly, depending on the year, region, and variety grown. Percentages of discolored seed ranged from 8 to 73 within certain States in the same year; and differences between States were even greater. In general, durums were more susceptible than bread wheats. *Alternaria* spp. were most commonly isolated, although *Helminthosporium* and

Fusarium were most prevalent in some regions in some years. In general, prevalence of seed discoloration was correlated positively with prevalence of microorganisms and plumpness of seed, and negatively correlated with percentage of germination and seedling stand. There was no association with test weight or with varietal reaction to leaf and stem rust. The benefits of seed treatment depend on the prevalence of *Helminthosporium* and *Fusarium* rather than on the total microflora. Seed treatments with Semesan Junior increased stands in certain lots from 23 to 67 per cent. (Cooperative investigations between the Division of Cereal Crops and Diseases, U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station.)

Boron Soil Treatments and the Development of Flax Diseases. HART, HELEN, J. E. MITCHELL, AND DAVID GOTTLIEB. The addition of boron to soil did not affect development of flax rust and pasmo in numerous field and greenhouse experiments in 1942 and 1943, although promising results in reducing rust had been obtained by other investigators in 1941. The application of 50 lb. or more of sodium tetraborate to sandy loam soil at University Farm, St. Paul, tended to reduce stands, height of plants, and yields of flax. The addition of even smaller amounts tended to increase damage from wilt and Rhizoctonia.

Effect of Zinc Sulphate-Lime on Protective Value of Organic and Copper Fungicides Against Early Blight of Potato. HEUBERGER, J. W., AND T. F. MANNS. Preliminary evidence indicated that the addition of zinc sulphate-lime to fungicides might aid in decreasing run-off and in increasing initial deposit and tenacity. In an experiment on Dakota Red potatoes, designed to evaluate the protective value of organic and copper fungicides, zinc sulphate-lime at 1–½–100 was added to 3 of 7 materials. Fungicides used were Spergon (tetrachloro-parabenzoquinone), Fermate (ferric dimethyl dithiocarbamate), U. S. Rubber Co. #604 (2, 3-dichloro-naphthoquinone-1,4), Rohm and Haas Co. He175 (disodium ethylene bisdithiocarbamate), Yellow Cuprocide (copper oxide), Compound A (copper oxychloride) and Bordeaux at a toxicant concentration of 1½–100. Seven applications at 200 gallons/acre were made at approximately 10-day intervals (July 29–Oct. 3) on replicated randomized plots. Records on Oct. 16 showed following control: He175-$ZnSO_4$-CaO, 92%; Yellow Cuprocide-$ZnSo_4$-CaO, 86%; Bordeaux, 85%; Compound A-$ZnSO_4$-CaO, 82%; Yellow Cuprocide, 68%; Compound A, 58%; #604, 54%, He175, 40%; Fermate, 15%; Spergon, 4%; unsprayed, 0%. The possibility of synergism, and of new compound formation in the He175-$ZnSO_4$-CaO combination, is being considered. (Yield records had not been taken when this abstract was submitted.)

Effect of Organic and Inorganic Seed Treatments on Rate of Emergence, Stand, and Yield of Soy Beans. HEUBERGER, J. W., AND T. F. MANNS. The following materials were used in a test on the Scioto variety of soy beans to determine if seed treatments were beneficial under Delaware conditions: Spergon (tetrachloropara-benzoquinone); Arasan (tetramethyl thiuram disulphide); Dow #5 (tetrachloroquinone); Dow #6 (2, 4, 5-trichlorophenate); Ceresan (ethyl mercury phosphate). Semesan (hydroxymercurichlorophenol); Yellow Cuprocide (copper oxide). The first 5 were use at 2 oz.–1 bu. and the last 2, Ceresan and Cuprocide, at 1 oz.–1 bu. Treatments were planted in replicated randomized plots on June 25. The percentage stand 5 and 15 days, respectively, after planting was: Dow #5, 46.8% and 86.1%; Arasan, 40.4% and 84.9%; Ceresan, 38.5% and 82.6%; Spergon, 37.8% and 85.2%; Dow #6, 29.8% and 75.0%; Semesan, 28.4% and 64.1%; Check, 28.1% and 65.0%; Yellow Cuprocide, 15.4% and 55.5%. These data show that the materials fell into three classes: (A) accelerated rate of emergence and increased final stand (Dow #5, Arasan, Spergon, Ceresan); (B) Little or no effect on rate of emergence and final stand (Dow #6, Semesan); (C) decreased rate of emergence and final stand (Yellow Cu ɔrocide). Yield data showed that materials in Class A increased yield, that those in Class B had little or no effect, and that those in Class C reduced yield.

Fire Blight Control by Prevention of Infection. HILDEBRAND, E. M.

The Problem of Dry Rot Caused by Macrophomina phaseoli (= Sclerotium bataticola). HOFFMASTER, D. E., J. H. MCLAUGHLIN, W. WINFIELD RAY, AND K. STARR CHESTER. Macrophomina dry rot ("charcoal rot") is a major pathological and economic problem in corn, sorghums, potatoes, beans, cowpeas, and peanuts in the South. Losses reach 48% in corn and 5–75% in potatoes. New hosts are catalpa, cedar, alfalfa, sudan grass, mung beans and broomcorn. *M. phaseoli* mainly attacks seedlings, causing damping-off, and sub-mature plants, the ensuant root decay resulting in stem-rot, precocious ripening, low yields, and premature death. It is moderately and variably aggressive. Its invasion is favored by devitalization, characteristic of plants subjected to environmental extremes of continental climates, and wounds or attacks of other organisms. It is adapted to high temperatures. The correct name, according to International Rules of nomenclature, and

that now generally accepted by American and British workers, is *Macrophomina phaseoli* (Maubl.) Ashby. Single conidia invariably produce the sclerotial stage. A theory that sclerotia may be immature pycnidia is proposed. Tentatively proposed control methods include cultural practices that increase crop health and vigor, liming of soil for sorghums, increasing the organic matter in soil, and use of resistant crop varieties, which include many sweet sorghums, some kafirs, and certain other sorghums. Resistance is characteristic of some corn hybrids.

Experimental Hosts of Tobacco-etch Virus. HOLMES, FRANCIS O. Tobacco-etch virus was transmitted to one or more species in each of 15 families of plants. Among the experimentally susceptible species were several that conceivably might function as overwintering hosts in nature. One of these, a perennial weed, *Physalis subglabrata*, was shown to be naturally infected in New Jersey. Although tobacco-etch virus is serologically and immunologically distinct from tobacco-mosaic virus, as well as much more sensitive to inactivation by heat, a marked correlation was found in regard to responses of plants to the two viruses. The species that proved susceptible to infection by tobacco-etch virus proved susceptible to infection by tobacco-mosaic virus, although the converse was not true.

Spraying Efficiency of New Types of Spray Equipment. HOPPERSTEAD, S. L., M. W. GOODWIN, AND PAUL L. RICE. The ability of the Speed Sprayer to apply a uniform deposit of spray materials throughout all parts of trees was compared to standard orchard equipment using two 8-nozzle brooms, one operated from the tower and one from the ground, and to a vertical boom attachment using adjustable guns. The vertical boom attachment was in the experimental stage. Twenty-three-year-old Stayman trees were used to which 12½ to 13 gal. of spray mixture were applied per tree per application. Seven spray applications were made. Leaf samples were taken before and after each application, and laboratory analyses made. Data obtained from analyses of samples collected one month after the final spray was applied were as follows: Speed Sprayer—top 213.1, bottom 296.0; Vertical Boom—top 313.7, bottom 297.5; Eight nozzle brooms—tops 269.3, bottoms 278.2, Nonsprayed trees 35.4. Results are expressed as micrograms As_2O_5 per sq. in. leaf surface. In addition, samples taken in the tops of the trees within a 5-ft. radius of the trunk gave the following analyses: Speed Sprayer—171.6, Vertical boom—262.3, eight nozzle brooms—185.0. Fruit records collected throughout the season and at harvest indicate close correlation between codling moth control and the leaf analyses. Fruit records were not completely assembled when this abstract had to be submitted.

The Coverage Factor in Fungicidal Efficiency. HORSFALL, JAMES G., AND A. D. MCDONNELL. The dynamics of coverage by protectants is not well understood, but a knowledge of them is currently urgent because of the possibility of extending critical fungicides by improving coverage. An effort was made, using the dosage-response technique, to distinguish the probability of hitting a spore (coverage) from the probability of killing it with what hits it. Statistically, a steep dose-response curve means high uniformity. Theoretically, the steeper the slope, the smaller the probability of missing the spore and the more nearly the experiment measures only the probability of killing the organism with what hits it. Field slopes are flatter than laboratory slopes presumably because the probability of missing the spore on a plant is greater than when the spore is placed on a sprayed slide. Laboratory slopes, however, can be steepened and performance of deposits can be improved by increasing spray time. In many but not all field trials on potatoes and tomatoes, slopes were steeper for long than for short spray times. Since run-off increases in the field with spray time, it tends to cancel the practical benefit of the improved coverage by the deposits that are actually retained by the foliage.

Cationic Phenyl Mercury Compounds as Specific Apple-scab Eradicants on Foliage. HOWARD, F. L., AND M. B. SORRELL. Two compounds excelling liquid lime sulphur for eradicating *Venturia inaequalis* hyphae and conidia from fruit and leaves without necrosis of healthy apple cells have proved promising in orchard, individual leaf, and laboratory tests. Phenyl mercuric 2′, 2″ nitrilo triethanol lactate (Puratize N5–X) and phenyl mercuric 2′, 2″ nitrilo triethanol acetate (Stearonyx P) are effective at dilutions of 1: 5,000 to 1: 10,000; inexpensive; available in quantity; compatible; and almost equally toxic in acid or alkaline water. In an orchard test of 7 fungicides with spray applications timed according to Rhode Island spray-schedule recommendations, a foliage index on July 20 or 10 randomized replicate trees showed 2% infection on McIntosh and 4% on Baldwin compared with 92% and 34% on respective control trees. A second index on September 2 showed no infection on McIntosh and Baldwin foliage in contrast to 74% and 20% on the respective control trees. Fruit exposed to the Puratize spray were free of Scab. Study of infected attached apple leaves, individually sprayed with the phenyl mercury compounds at dilutions of 1: 5,000 and 1: 10,000, indicates that only the pathogen

and injured suscept cells are killed and not healthy apple tissues. Laboratory toxicity tests against *Macrosporium sarcinaeforme* spores show an L. D. 50 at 3 p.p.m. and a steep slope "dosage response" curve.

Cationic Quaternary Ammonium Compounds as Fungicides. HOWARD, F. L., AND H. L. KEIL. The fungicidal properties, phytotoxicity, and detergency of the generic group of chemicals having the type formula

$$\begin{matrix} R\,1 \\ R\,2 \\ R\,3 \\ R\,4 \end{matrix} \!\!\diagdown\!\!\diagup\!\!\diagdown\!\!\diagup\!\!\diagdown\!\!\diagup\

size of fruit. Dilute Bordeaux, or Bordeaux substitutes, tried in 4-spray programs, (1) just after petal-fall, (2) 10 days or 2 weeks later, (3) 2 weeks later, and (4) just after harvest, have not given adequate control in years of severe epidemics. Epidemiological studies and chemical determinations of spray residues indicate that a critical period for control comes between the first and second applications. In 1942 a severe outbreak of the disease occurred in well-managed orchards when a protracted, heavy rain period slightly delayed the second application. Experiments were initiated on programs of graduated dosage in the several applications. In 1943, a season of severe leaf spot occurrence, various programs of this type (*e.g.*, Bordeaux (1) 6–8–100, (2) 3–4–100, (3) 1½–2–100, (4) 6–8–100) gave satisfactory results in disease control. Other data suggest 3–4–100 Bordeaux would have sufficed in application 4. The crop was too sparse for adequate data on fruit size.

A Foliar Mottle and Necrosis of the Chippewa Potato Associated with Infection by a Strain of the Potato x Virus. LARSON, R. H. A mosaic of Chippewa characterized by irregular chlorotic-mottle patches in intercostal areas of upper leaves and small scattered necrotic-flecks on older leaves was observed in the field in 1942 and 1943. External and internal root, stem, and petiole tissues of affected plants are normal. Tubers are slightly smaller than normal, but show no pathologic symptoms. Aphid (*Myzus persicae* and *Macrosiphum solanifolii*) transmission tests yielded negative results. Artificially inoculated *Nicotiana tabacum* and *N. rustica* developed small, local ring spots, systemic ring spots and irregular line patterns. Potato seedling 41956 was immune in all tests. Complete protective action of a common mild latent-mottle strain of the potato x-virus was shown on *N. tabacum, Datura metel* and *D. meteloides*, indicating the virus is a variant of the x-virus group. It would appear, therefore, that Chippewa, highly resistant to mild mosaic and a masked carrier of common strains of the x-virus, is subject to infection by an uncommon strain of the latter, which causes a mosaic mottle and necrosis. Infection was secured on Chippewa, Sebago, Triumph, Red Warba and Russet Burbank and on first-generation potato seedlings derived from seed of Katahdin by selfing. Symptoms developed more rapidly and severely at 20° C. than at 24° C., while they were entirely suppressed at 28° C. and above.

Studies on Ustilago striaeformis from Bluegrass. LEACH, J. G., AND C. V. LOWTHER. Spores of *U. striaeformis* from mature sori on bluegrass are very difficult to germinate, and artificial inoculations with such spores have produced low percentages of infection. By using spores from young unruptured sori and mycelium in tissues just prior to spore formation, the fungus has been isolated in pure culture on agar. Both sporidial and mycelial growth types occur. Certain growth types produce chlamydospores in great abundance. Spores produced in culture are slightly larger than those produced on plants and less prominently echinulate. A few are abnormal in shape. Spores produced in artificial culture have not yet been germinated successfully. The cytology and infection capabilities of the various cultural types are being studied.

A Method for Developing an Epiphytotic of Ergot. LEWIS, RALPH W. In order to induce an epiphytotic of ergot an abundance of spores was so prepared that the spores remained viable in cold storage and on the plant following application. Cultures were grown on sterilized wheat. When the spores formed, the cultures were beaten in a blendor with a minimum of water and screened. An equal weight of beet sugar was added to the thick spore suspension. Tests determined that spores prepared in this way remained viable in cold storage for 3 to 4 months and withstood the drying action of the air for days without a great loss in germinability. The spores germinated on slides when drops of the original or dried suspension were diluted with water. Spore suspensions were applied to rye flowers in bloom with a hand or motor sprayer. 59% of the heads became infected in a plot sprayed 5 times with a knapsack sprayer and 33% became infected in plots sprayed 3 times with a power sprayer. Adjacent control plots were 10% and 3% respectively.

Fermate Offers Promise in the Control of Frog-eye Leaf Spot (Sphaeropsis malorum) of Apple. LYLE, J. A., AND LUTHER SHAW. Results of the 1943 season indicate that ferric-dimethyldithio-carbamate (Fermate), used at a 3–5–50 concentration, offers promise as a control of leaf spot (*Sphaeropsis malorum*) of apple. Other fungicides applied included different concentrations of Bordeaux mixture, Tennessee Copper "26," Cupro-K, copper oxychloride, and silver nitrate. Two leaf-spot counts were recorded, the first in the middle of the season, the other during harvest. At each count 500 leaves per tree were examined. Each plot, containing 2 trees per plot, was replicated at least twice. The Fermate plots manifested a total of 10.7 per cent leaf infection for the season with no defoliation. The remainder of the sprayed plots ranged from 41.4 per cent infection for 4–4–50 Bordeaux mixture, to 66.1 per cent for 3–3–50 Tennessee Copper "26" with

some defoliation. The check plots had 100 per cent infection at both counts with half defoliation at the end of the season. It is significant that the Fermate plots exhibited leaves infected with only 1 to 3 lesions per leaf, whereas the other sprayed plots manifested 65 per cent of the leaves with more than 4 lesions. Effective control was not obtained from this material against either bitter rot or blotch of the fruit.

Natural and Mechanical Injury in Flax in Relation to Seed Treatment. MOORE, M. B., AND J. J. CHRISTENSEN. A study was made of the relation of seed injury of flax to differential response to seed treatments. Three kinds of injury were of frequent occurrence: mechanical cracking and shattering of the seed coat; discoloration and roughening of the seed coat due to fungi and weathering; and exposure of the embryo due to a natural tendency of seed of certain varieties to split at the germ end. Of the three, embryo exposure was the most common. There was no association between types of injury, but golden-seed varieties were much more subject to embryo exposure than brown-seed ones. Seed lots from Bolley Golden Selection C.I. 976 grown in 31 localities in the United States and Canada differed strikingly in percentage of cracked seed (0–32), of seed with exposed embryos (6–83), and of discolored seed (0–50). The differences in amount of injury between 7 varieties in a single locality were sometimes almost as great as those between seed lots of a single variety grown at different localities. In general, injured seed lots tended to respond to seed treatment more than non-injured; and golden-seed varieties responded more frequently than brown-seed ones. Ceresan and Arasan were about equally effective in increasing the stand.

Sunscald as a Predisposing Factor to Soft Rot of Early Potatoes in Transit. NIELSEN, L. W. The early potato crop of North Carolina is harvested during late May, June, and early July. On clear days exposed tubers may be seriously sunscalded. Tubers exposed on the surface of the ground have developed temperatures of 52.2° C., and in bags on open trucks, 50.5° C. Tubers exposed on the ground for 6 hours on July 10, 1942, had bacterial soft rot in excess of 50 per cent after 6 days storage. Potatoes exposed July 21, 1943, for 0, $1\frac{1}{2}$, $2\frac{1}{2}$, $4\frac{1}{2}$, and $5\frac{1}{2}$ hours had the following percentages of rot after storage for 14 days: 3.57, 10.1, 28.0, 74.5, and 78.5, respectively. Using the rapid growth of soft-rot bacteria on cooked potato tissue as an indicator of heat injury, it was found that Irish Cobbler tubers were injured when exposed in a water bath at the following intervals and approximate temperatures: 10 minutes, 51° C.; 30 minutes, 45°–47° C.; and, 60 minutes, 43°–45° C. A tuber (3.0 inches in diameter) exposed to artificial light reached 44.7° C. on the irradiated side after $1\frac{1}{2}$ hours, and was injured to one-third its thickness. In many cases the injury could not be visually detected. These data suggest that some of the soft rot developing in transit is traceable to heat injury prior to packing.

An Eradicant Treatment for Sooty Blotch of Pears. PALMITER, D. H. The results of field experiments indicate that a greentip application of Elgetol to Kieffer pear trees will sufficiently reduce the sooty-blotch fungus, *Gloeodes pomigena,* to permit a commercially clean crop of fruit without the aid of summer applications of fungicides. Trees that received a 1% Elgetol application in 1942 produced a crop 80% clean, 13% slightly blotched, and 7% heavily blotched compared with 96% blotched fruit on nonsprayed trees. In 1943 the same treatment, applied to different trees, resulted in 96% clean fruit and 4% slightly blotched fruit. Fruit on nonsprayed trees were 80% infected. Lower dosages considerably reduced the incidence of the disease and may prove sufficient under some conditions.

Arborvitae Blight. PLAKIDAS, A. G. A disease of oriental arborvitae (*Thuja orientalis*), referred to locally as "blight" or "fire," has been known in the South for many years. Its chief symptoms are dying and browning ("firing") of leaves, small twigs, or entire branches, and often complete killing of the entire tree. The disease has been attributed to red spider, drought, excessive summer heat, and winter injury. The varieties Berkmann's Golden and Baker are most severely affected, but the disease occurs also on many others. Similar symptoms also occur on the Italian Cypress (*Cupressus sempervirens*). A fungus, apparently a *Cercospora,* has been found constantly associated with the disease. Inoculations with pure cultures have produced it in typical and severe form. Infection also has been obtained by placing conidia-bearing twigs on healthy plants. The fungus has been reisolated from the inoculated plants and resembles somewhat *Cercospora sequoiae* and *C. sequoiae* var. *juniperi,* but differs from these in length of conidiophores and rate of growth in culture. It probably is a distinct species. Perithecia of *Mycosphaerella* sp. are often found on diseased twigs, either alone or in association with *Cercospora.* Ascospore isolates appear similar to *Cercospora* isolates in culture, but inoculations with these have not produced infection thus far.

Use of a Ferric Dimethyldithio Carbamate and Talc Dust to Combat the Phomopsis Blight of Eggplant. PORTER, RICHARD P. A single season's field results indicated that

a 10–90 Fermate (Ferric dimethyl dithio carbamate) talc-dust treatment was slightly more effective than a 20–80 copper-lime dust in controlling the leaf-spot symptoms of Phomopsis blight. During the early onslaught of the disease it gave significantly better protection in preventing fruit rot. The plants treated with the Fermate dust also outyielded those dusted with copper-lime.

The Incidence of Apple Scab and Cedar Rust on Wealthy Apples and Their Effect on Fruit Development in Minnesota. SHARVELLE, E. G. Apple scab and cedar rust are the most important apple diseases in Minnesota. During the summer of 1943 a maximum fruit infection by scab of 92 per cent and a maximum fruit infection by cedar rust of 60 per cent were recorded on Wealthy apples; however, the introduction of a new spray schedule and spray timing service resulted in controlling all of the major diseases and insect pests. Detailed studies were made on the effect of apple scab and cedar rust on the size and weight of apples. Severe scab caused an average reduction of 20 per cent in size and of 43 per cent in weight. Very light infection by scab caused a reduction in size of approximately 8 per cent. Cedar-apple rust was found to cause an average reduction in size of approximately 8 per cent and a reduction in weight of infected fruit of 24 per cent.

Cultural Variation in Single Ascospore Isolates of Sclerotinia fructicola (Wint.) Rehm from Cherry Plum Hybrids. SHARVELLE, E. G., AND SHAN-MING CHEN. Attempts to isolate complete sets of ascospores from single asci of *S. fructicola* were unsuccessful until the conditions influencing ascospore discharge were determined. Maintaining the ascus material at 15° C. for 4–10 hours, then transferring to room temperature for two hours, appeared to stimulate ascospore discharge. Sixteen lines obtained from 2 asci fell into 4 distinct groups on the basis of cultural characteristics, sensitivity to sulphur fungicides, and pathogenicity on apple fruits.

Use of Potato Juices for Cultivation of Corynebacterium sepedonicum. SNIESZKO, S. F., AND REINER BONDE. Potato filtrates were prepared by expressing juice of mature tubers or stems and leaves. Some juices were prepared after preliminary freezing of tissues; others were treated with sulphites to prevent darkening of the juice. The juices were first centrifuged and then sterilized by fitration through the Seitz No. 1 sterilizing filter sheets. For the cultivation of *C. sepedonicum* the filtrates were used alone, or with addition of media, phosphate buffers, or agar. The filtrates obtained from the potato varieties Katahdin, Green Mountain, Sebago, and President supported abundant growth of *C. sepedonicum.* Less satisfactory as media were varieties believed to be resistant to ring rot and designated as U. S. seedling varieties, .055, 47102, and 886. Two U. S. seedling varieties, *i.e.*, 46952 and 870, supported little or no growth of *C. sepedonicum.* Results indicated some coincidence between resistance of the potatoes to ring rot and abundance of growth of *C. sepedonicum* on filtered potato juices; the variety President seems to be an exception. There apparently is little if any correlation between the pH of the juices and the growth of *C. sepedonicum.*

Races of Puccinia graminis avenae in the United States. STAKMAN, E. C., M. N. LEVINE, AND W. Q. LOEGERING. Although races 1, 2, 5, 7, 8, 10, and 12 of *Puccinia graminis avenae* have been found in the United States, only the first three have heretofore been present in important amounts. Consequently, the oat varieties Richland, susceptible only to 8 and 10, and White Tartar, susceptible only to 7 and 12, have been generally resistant in this country and were, therefore, used as resistant parents in a breeding program. In 1940 and 1941, however, there were indications of increasing prevalence of races 8 and 10; and in 1943 race 8, widespread geographically, comprised about 12 per cent of all racial isolates, and caused appreciable rust on Vicland, Boone, Tama, and other hitherto resistant varieties, mostly derived from Victoria × Richland crosses. This race and the other dangerous races, 7, 10, and 12, were found first in barberry-infested areas. Race 8 may or may not become abundant in the near future, depending on conditions affecting its persistence and spread. Certain generally virulent races, notably 4 and 6, have not been found in the United States, although reported occasionally from Canada. (Cooperative investigations between the U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station.)

Comparison of Soil Fumigants for the Control of Root-knot Nematode. STARK, F. L., JR., A. G. NEWHALL, AND BERT LEAR. The efficacies of several liquid nematocides were compared in a commercial greenhouse by injecting the fumigants 4 in. deep, 10 in. apart in replicate plots. In fall treatments of heavily infested soil ethylene dichloride, a mixture of methyl bromide-ethylene dichloride-carbon tetrachloride (1–6.5–2.5) (Dowfume Br–10), and chloropicrin (Larvacide) all gave highly significant control of nematodes and increased yields on a spring crop of tomatoes as compared to checks and a furyl nitro-

ethylene dust treatment. The methyl bromide mixture at 5 cc. per injection gave better control of nematodes than chloropicrin at 2 cc. and ethylene dichloride at 15 cc., but yields were about the same for each treatment.

In a set of spring treatments on soil less heavily infested chloropicrin-methyl bromide mixture (1–3), chloropicrin, methyl bromide-ethylene dichloride-carbon tetrachloride mixture, and chloropicrin-ethylene dichloride mixture (1–9) in descending order all significantly reduced nematode injury. However, there was no significant difference in the yields.

When 3, 5, and 7 cc. dosages of the methyl bromide-ethylene dichloride-carbon tetrachloride mixture were compared, it was found that increasing the dosage markedly reduced nematode injury, but the effect on yield was scarcely significant.

It is concluded that where nematodes are severe all treatments will give an economic net return. At the rates employed treatments can be listed in ascending order of their cost as follows: methyl bromide-ethylene dichloride-carbon tetrachloride mixture, ethylene dichloride, chloropicrin-ethylene dichloride mixture, chloropicrin, and methyl bromide-chloropicrin mixture.

The Coverage Effect of Sulphur on the Control of Apple Scab. STODDARD, ERNEST M., AND WENDELL D. HENRY. The effect of coverage on control of apple foliage scab was studied by applying four dosages of sulphur per tree in 2 gallonages. The per cent of infection, plotted as probits, showed a linear relationship to the logarithm of the dose of fungicide. Doses applied in high gallonage gave a steeper slope than those applied in low gallonage. Statistically, a steep slope suggests low variability, or in this case better coverage of each unit of leaf area. This should improve the probability of hitting any given spore. With different slopes, the curves crossed, so that the largest dose per tree gave better control when applied as low concentration-high gallonage, and the smallest dose per tree gave better control when applied as high concentration-low gallonage. At the highest dose, therefore, the probability of hitting a spore with the high gallonage-low concentration seems to be of greater consequence than the probability of killing the spore with high concentration-low gallonage. At the lowest dose, on the other hand, the probability of killing the spore with the high concentration-low gallonage is greater than the probability of hitting it with high gallonage-low concentration.

Fermate for Control of Early Blight on Tomato. TAYLOR, CARLTON F., W. H. CHILDS, AND J. G. LEACH. As a tomato spray in 1942, Fermate (ferric dimethyl dithiocarbamate) at 2 lb.–100 gal. proved superior to 80% tetramethyl thiuramdisulphide (2–100), wetable Spergon (2–100), Cuprocide (1½–100), and Bordeaux mixture (8–8–100) in preventing foliage injury caused by *Alternaria solani.* Yield data were limited to the weight of green fruit remaining when the foliage was killed by frost, fermate having twice the yield of any other treatment at this period.

Fermate, diluted in Pyrax AAB, was tested as a dust on tomatoes in 1943. Data based on individual-plant estimates rated the percentage of green foliage in early October in descending order as follows: 10% Fermate, 5% Fermate + 10% Sulphur, 5% Fermate, 2.5% Fermate, and 6% copper oxychloride sulphate. Yield differences in ripe fruits were non-significant throughout the season, but the quality of the fruit was noticeably better on the plots with less foliage injury.

The Fermate–Pyrax AAB mixture is mechanically well-adapted to dust application.

The Influence of Known Chemicals on the Initiation of Pathological Growth and Symptoms Resembling Those from Certain Viruses. THOMAS, JOHN E., AND A. J. RIKER. The effect of 31 known chemicals has been studied on sunflower, marigold, tomato, Paris daisy, velvet leaf, *Kalanchoe,* and *Bryophyllum.* The chemicals were commonly mixed in a lanolin paste and applied to the decapitated stem tips of plants 1½ to 2 inches above inoculations with the attenuated (A6–6) strain of the crown-gall organism, *Phytomonas tumefaciens.* The results from over 2300 chemical treatments and over 4800 inoculations indicated that 12 of these compounds could more or less regularly "activate" tissue about the attenuated bacteria. The most active compounds were indole-butyric acid, alpha-naphthylacetamide, and beta-naphthoxyacetic acid. Other active compounds were p-chlorophenoxyacetic acid, alpha-naphthalene-acetic acid, indole-3-aetic acid, indole-propionic acid, alpha-naphthalenepropionic acid, tryptophane, 2-chloro-5-nitrobenzoic acid, 2-bromo-3-nitrobenzoic acid and phenoxyacetic acid. In general, the other common growth-substance responses noted included axillary bud and abscission layer inhibition, aerial root stimulation, stem thickening, epinasty, gall formation and leaf distortions. Some leaf malformations appeared very similar to certain ones induced by viruses, but they were not transmissible and plants eventually recovered. Variations in growth substance responses were found with different hosts and with different chemicals.

The Bacteriophage Reaction as a Means of Quick Identification of Pathogenic Bacteria. THOMAS, R. C. The need for a quick identification method to confirm the presence

of a bacterial plant pathogen frequently has been felt. A study made of 10 plant and 11 human pathogens, as well as of several saprophytic forms of bacteria, indicates that the phage reaction can be used for identification purposes. A phage was prepared for each species of bacteria and tested against all of the other species. Phages appear to show as much specificity of reaction as do diagnostic sera. A few interesting relationships among species were revealed, which suggest further study.

Tobacco Leaf-spot Bacteria on Roots of Pasture Plants. VALLEAU, W. D., E. M. JOHNSON AND STEPHEN DIACHUN. Earlier studies have shown that *Bacterium tabacum* and *B. angulatum* multiply on the roots of several crop plants and weeds and may overwinter in this way on the roots of cover crops planted after tobacco. More recent studies demonstrate that *B. angulatum* may persist on the roots of cover-crop plants (wheat and crimson clover) for at least 21 months after tobacco harvest. Inoculations with soil cores containing roots from bluegrass fields in which tobacco beds were prepared proved that the organisms were present, presumably on the roots of some pasture plants, before the diseases appeared in the plant bed. *B. tabacum* also was obtained from weed roots in a field, prepared for setting tobacco, at setting time.

Charcoal Rot of Irish Potatoes. WATSON, R. D. Irish potatoes in Eastern Texas often rot in the ground or in storage. This condition was particularly severe in 1943, with losses of 15 to 20 per cent of the crop; some growers lost more than half their crop.

During the past season, the primary causal agent of this tuber rot proved to be *Sclerotium bataticola.* The fungus often enters the tuber at the stolon end. Eyes and enlarged lenticels are also points for infection. The diseased stolon is ashy-grey due to embedded sclerotia. In the tuber the fungus produces a semi-wilted, somewhat watery black rot, which is generally shallow. If no secondary infections occur, the tuber develops into a leathery mummy. This seldom occurs, since one or two secondary rots usually help complete the tuber breakdown. A soft rot, caused by *Erwinia carotovora,* and a dry rot (*Fusarium* sp.) are secondary rots associated in the final stages with charcoal rot.

The losses are closely correlated with high temperatures and high soil moisture—the chief epiphytological factors.

Effectiveness of Monocalcium Arsenite as an Eradicant Spray Against Sclerotinia laxa. WILSON, E. E.

The Chlorophenates as Eradicant Sprays Against Sclerotinia laxa and Coryneum beijerinckii. WILSON, E. E. The major weakness of sodium tetrachloro and pentachlorophenates as eradicant sprays against *S. laxa* and *C. beijerinckii* appears to be their failure to destroy the mycelium of the fungi inside the host tissue. Although the materials may destroy most of the conidia the mycelium produces new conidia within 2 or 3 weeks. Applications of the sprays as near as feasible to the critical periods for infections (blossoming and leaf-production stages of the host) lessens the chances for the fungi to produce a timely supply of inoculum, but increases injury to the host. Addition of an emulsible oil and a wetting agent increased the penetration of the chlorophenates into host tissue, and to some extent improved their eradicant effect. In field tests on almond trees, sprays composed of 6 pounds of these materials to 100 gallons of water plus 0.5 per cent oil and 8 oz. to 100 gallons of a wetting agent, destroyed from 77 to 97 per cent of the sporodochia of *S. laxa* and prevented immediate production of a new crop of conidia. The effect on the disease was not observed because of blossom injury. The same sprays reduced leaf infection by *C. beijerinckii* 99 per cent. Copper salts of trichlorophenate and tetrachlorophenate were less effective against both fungi than the sodium salts.

Control of Tomato Anthracnose. WILSON, J. D. All copper-containing fungicides are comparatively ineffective in anthracnose control. Fermate may give a high degree of control but must be properly used as to timeliness, concentration, rate of application per acre, etc. It is very important that the first application be made early enough, *i.e.,* about July 20 to 30 for cannery tomatoes in northern Ohio, or when fruits of the first cluster are approximately half-grown.

Protection with Fermate continues as long as 40 days after the last application, in early schedules. Since fruits ripening in late September were pollinated approximately 50 days earlier, it seems possible that protection may result from absorption of something from the Fermate by the host plant. Other possibilities are that the fungicide reduces the inoculum present in the surface soil and in fruit lesions, or, less likely, that the fungus is kept from foliage and stems of the tomato plant.

Detached fruits and fruits on defoliated plants are highly susceptible to infection. Sprays that injure the host plant promote fruit infection. Dusts seem as effective as sprays in checking fruit loss. There is some variation in varietal susceptibility, but all commercial varieties used experimentally have shown heavy infection under certain field conditions.

The Present Status of the Fixed Coppers as Fungicides. YOUNG, H. C. During the past several years a study has been made of many fixed copper compounds to determine their role as fungicides. Results of laboratory and field tests indicate that considerable variation exists in both effectiveness and injury. The general groups, used in these comparisons, were: (1) the basic copper chlorides, (2) the basic copper sulphates, (3) the copper oxides, and (4) a miscellaneous group.

It was found that the basic chloride and oxide groups were, in general, somewhat more injurious to foliage than the basic sulphate or several of the miscellaneous group, but were also somewhat more effective as fungicides. It also was found that most fixed copper compounds adhered to foliage poorly, which factor may be responsible for the moderate effectiveness of the sulphate group. Many diluent and adhesive materials have been tried but no generally effective one was found.

There is a tremendous variation in the particle size (1 to 75 microns) in the various commercial fixed-copper products. This factor alone influenced adhesiveness and also handicapped the standardization of dusts and their application. It was found that the micronization of a commercial tribasic copper sulphate greatly improved it fungicidally, as well as its physical condition for application.

Factors Influencing the Production of Local Lesions by the Potato Yellow-dwarf Virus on Leaves of Nicotiana rustica. YOUNKIN, S. G. It has been shown that variations in environmental conditions, size of inoculated leaves, and inoculation procedures may influence the number, disposition, size, and appearance of the local lesions induced by the potato yellow-dwarf virus on mechanically inoculated leaves of *Nicotiana rustica.* Optimum temperature for local lesion production was 75 to 80 degrees F. At lower temperatures the time required for lesion development was excessive. At higher temperatures the size and character of the lesions were altered. Maximum numbers of local lesions resulted when plants were placed in complete darkness for 12 hours immediately after inoculation. Leaves of *N. rustica* produced by rapidly growing plants and measuring 9 cm. × 13 cm. yielded more local lesions than either (1) leaves measuring 8 cm. × 12 cm. and less or (2) those measuring 13 cm. × 17 cm. and more. Uniform distribution of lesions over the inoculated leaf surface was obtained only with leaves of optimum size. Application of 600-mesh carborundum to the leaves, before inoculation, increased the yield of local lesions. Washing of leaves immediately after inoculation failed to affect the number of local lesions when inoculum consisted of crude sap from infected plants that had been diluted with water.

Mechanism of Action of 8-hydroxyquinoline. ZENTMYER, GEORGE A. 8-hydroxyquinoline has long been known to have bacteriostatic and fungistatic properties, and is extensively used as an antiseptic. The mechanism of action of this organic is, therefore, of interest. The fact that 8-hydroxyquinoline is a useful agent in quantitative analysis for precipitating many minor elements (Cu, Mn, Fe, Zn), suggested the theory that it acts fungistatically by precipitating one or several of these elements so that the microorganisms cannot use them. *In-vitro* experiments show complete inhibition of growth of several fungi (*Ceratostomella ulmi, Fusarium oxysporium, F. lycopersici, Penicillium* sp.) by 8-hydroxyquinoline at a concentration of 1–10,000, at pH 6. If the acidity is increased to pH 3, by 0.1 N HCl, growth of these fungi is unaffected by the presence of 8-hydroxyquinoline. When a culture growing at low pH (3.0), in the presence of 8-hydroxyquinoline, is filtered through a Berkefeld filter and the filtrate is readjusted to pH 6 and reinoculated with the fungus, inhibition of growth occurs. These findings are in agreement with the fact that the inner complex metal-hydroxyquinoline salts are known to be soluble in solutions of mineral acids. This cyclic organic chemical thus may be acting as an antivitamin, in the case of minor elements which are connected with vitamin formation and action.

Logarithmic-Probit Relation of Spore Dosage and Response in Dutch Elm Disease. ZENTMYER, GEORGE A., JAMES G. HORSFALL, AND PHILIP P. WALLACE. In general, biological responses follow the logarithm of the dosage. This principle has been applied in studies (1) on distance of local spread of the Dutch elm disease, and (2) on spore dosage in relation to disease incidence and to amount of wood discolored in inoculated elms. In (1), the percentage of trees diseased, plotted as probits, decreased with the logarithm of the distance from a central source of inoculum. In (2), dosages of 10, 10^2, 10^3, 10^4, 10^5, and 10^6 spores per tree were injected into elms; the disease response as percentage wilt per tree, plottd as probits for this "all-or-none" type of reaction, increased directly with the logarithm of the number of spores injected. In the graded type of response, the total length of wood showing vascular discoloration followed a linear relation to the logarithm of the number of spores introduced into the tree. The spore-dosage data also provide a sound explanation for the failure of the expected percentage of elms in heavily infected areas to show disease symptoms. The disease apparently will not build up sufficiently to develop symptoms when the tree receives only a small dose of spores.

STUDIES ON CYTOLOGY OF USTILAGO CRAMERI[1]

C. S. WANG

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INTRODUCTION

Ustilago crameri (smut of millet) is one of the few smuts known to complete its life cycle on artificial substrata (28); therefore, it affords an exceptional opportunity to study the nuclear behavior in saprophytic as well as in parasitic stages.

It has been shown frequently that new physiologic races of certain smut fungi may arise through hybridization or mutation (8, 19, 23). Nevertheless, many of the investigations have not dealt with the actual mechanism concerned in the behavior of the nuclei, that is, the cytological basis for the possible origin of new races. It is well known that meiosis in most smuts occurs during chlamydospore germination, but it is not known at what stages during germination the reduction of chromosomes takes place (10, 12, 23). Moreover the nuclear behavior of the hyphal cells in either parasitic or saprophytic stages varies in different species, and in the same species at different stages of development (15, 24, 25). Therefore, the writer investigated these phases of the problem as thoroughly as possible.

Practically, the resistance or susceptibility of the host to smut fungi is determined by the presence or absence of smutted kernels, but actually this is not proof that the fungus can or cannot penetrate into the host. In fact Brefeld (5, 6) and others (16, 20) thought that the hyphae of certain smuts grew so slowly that they were unable to reach the meristem before the surrounding stelar tissue hardened. Recently, from a histological study, Western (30) concluded that there are three different bases for resistance in oats smut: a reaction of the epidermal cell wall which prevents penetration, necrosis of the host cells, and a retarding effect on the growth of mycelium within the host. Since there is virtually no information on the relationship of *Ustilago crameri* to the host plant the writer also investigated this problem.

The literature on the cytology and host parasite relations in the smuts has recently been adequately reviewed (12, 13, 15, 22, 29, 30) and therefore will not be repeated here, but literature which is pertinent to particular phases of this study will be referred to whenever necessary.

EXPERIMENTAL RESULTS

Cytology of Ustilago crameri

Nature and Type of Chlamydospore Germination. The chlamydospores of *Ustilago crameri* Kcke. are ovoid to subspherical and 8 to 11 μ long.

[1] A portion of a thesis presented to the Graduate School, University of Minnesota, July, 1937, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Published as Paper 2086 of the Journal Series of the Minnesota Agricultural Experiment Station.

Under ordinary conditions the spores are capable of germination without dormancy and have been shown to remain viable for at least 62 years (27). On germination, a promycelium emerges through a gap in the spore wall. It may branch considerably, but no sporidia have been observed. Sometimes 2 or more promycelia may arise from a single chlamydospore.

Meiosis. In order to study the nuclear behavior during germination, spores were dusted with a camel-hair brush, on a thin film of 1 per cent malt agar or 1.5 per cent potato-dextrose agar, spread upon slides, then put in a sterilized moist chamber until the desired stage of germination had been reached. The material was then killed in Flemming's weak solution, washed, and stained in iron-alum haematoxylin.

The mature chlamydospore of *Ustilago crameri* contains a single nucleus (Pl. 1, Fig. 1), which is diploid. This also is generally true for other smut fungi (9, 17, 18, 24, 29). In *U. crameri* at germination, the first division of the diploid nucleus takes place either in the chlamydospore or in the promycelium (Pl. 1, Figs. 1 to 10). Unfortunately, the early stages of the prophase were not observed. Later, 4 small deeply staining units, which are probably the chromosomes, can be seen in the nuclear membrane, but the chromosomes are too small to allow one to see the actual split. Subsequently, the chromatin masses move apart and contract into 2 daughter nuclei (Pl. 1, Figs. 1 to 4). In the meantime a papilla appears with dense cytoplasm (Pl. 1, Fig. 4). The papilla, which has grown gradually toward the wall, then breaks through it and finally passes outward, forming the promycelium. At the same time the diploid nucleus migrates into the young promycelium and divides (Pl. 1, Fig. 7). The nuclear division in the promycelium is more clearly visible than that in the spore, but it still has not been possible to observe the early prophase of the division. The stages from late prophase to the end of the telophase are shown in plate 1, figures 7 to 11. A typical telophase figure with a single strand of fibers between the two daughter nuclei and the newly forming septum is shown in plate 1, figure 10.

The second division quickly follows the first; in fact, a resting stage is rarely found. In plate 1, figure 12, is shown a 2-celled promycelium with 2 nuclei, each nucleus containing 2 chromosomes (haploid number for *Ustilago crameri*) with a more or less indistinct nuclear membrane. The subsequent stages are shown in plate 1, figures 13 to 15. It is evident that in the prophase of the second division (Pl. 1, Fig. 12) only 2 chromosomes are shown, and at the end of this division there are 2 chromosomes in each daughter nucleus (Pl. 1, Fig. 14). It appears, therefore, that reduction in number of chromosomes has taken place at the first division. In contrast to this, the reduction occurs at the second division in the promycelia shown in plate 1, figures 16 to 18, as there are 4 chromosomes at the beginning of the second division (Pl. 1, Fig. 16). Variations in the chromosome conditions are shown in plate 1, figures 19 to 22, and 23 to 24. In figures 19 to 22, there apparently is no reduction in chromosome numbers either at first division or second division. Particular attention is called to figure 21, because

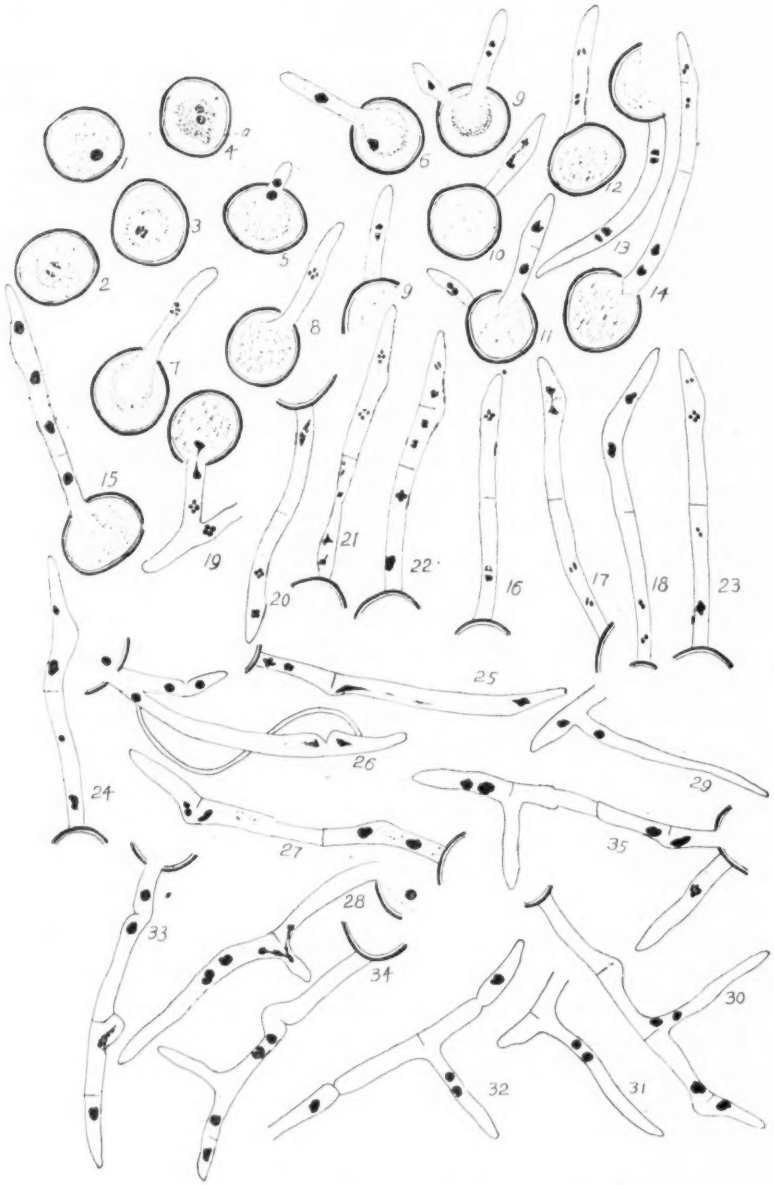

PLATE 1. Fig. 1. A mature chlamydospore with a diploid nucleus. Figs. 2–4. Different stages of the first division in the chlamydospores. In figure 4, *a* is a papilla. Figs. 5–6. Showing the migration of a nucleus from the chlamydospore to the promycelium. Figs. 7–11. Various stages of first division in the promycelium, showing the division figure. Figs. 12–14. Various stages of second division, showing reduction in chromosome number in first division. Fig. 15. A normal promycelium with four haploid nuclei, after two divisions. Figs. 16–18. Reductions in chromosome number in second division. Figs. 19–24. Abnormal reduction division. Figs. 25–32. Initiation of dikaryophase by "knee joint" between two adjacent cells, showing migration of nucleus. Figs. 33–34. Nuclear migration and association, slightly different from figures 25–32. Drawn free-hand; approximately × 1,000.

the nucleus is now in the third division, with 4 chromosomes still distinctly visible, and the spindle fibers are clearer than any others so far observed. In plate 1, figure 23, it will be seen that there are 2 nuclei with 4 chromosomes each and 2 with 2 each; consequently, there are 2 large nuclei alternating with 2 small nuclei in the same promycelium (See also pl. 1, Fig. 24).

The frequency of fusion between the 2 middle cells and the 2 end cells of the promycelium support cytological evidence that reduction in chromosome number may occur in either the first or second division. Therefore, in order to obtain more definite information on the frequency of segregation in either division, observations were made on promycelial cell fusions. Chlamydospores were germinated in potato-dextrose agar at 25° C.; after 72 hours, 300 counts were made on the frequency of cell fusion. The actual ratio of the middle to adjacent end cell fusion was 38.7 : 61.3 as compared with the theoretical ratio 37.5 to 62.5 (3 : 5) if the frequency of segregation in the first division and in the second division were equal.[2]

Bauch (2) and Hüttig (14) have shown that temperature has considerable influence on the time of chromosome reduction in certain smuts. Four different temperatures were tested for their effect on the time of chromosome segregation. The results are given in table 1 from which it can be seen

TABLE 1.—*Effect of temperature on segregation of factors for sex in Ustilago crameri, as indicated by the fusion of promycelial cells*

Temperature, ° C.	Fusion			
	Middle cells		Distal cells	
	Actual number	Per cent	Actual number	Per cent
15	115	37.8	189	62.2
20	118	41.0	170	59.0
25	130	38.7	206	61.3
30	121	38.9	190	61.1

that the ratio of middle cell fusions to adjacent end cell fusions is approximately 3 : 5 at all temperatures. This also indicates that the ratio of segregation of sex factors in the first division is about equal to that in the second division and that the ratio was not modified materially by temperature.

Origin of Haploid Colonies

It is difficult to isolate haplonts in *Ustilago crameri* because no sporidia are produced. The writer attempted to obtain haploid lines by the method described by Christensen (7) for *Ustilago tritici* (Pers.) Rostr. To isolate haploid lines of *Ustilago crameri*, single chlamydospore cultures were made on hanging drops. As soon as the cultures could be seen with the naked eye,

[2] If segregation for sex factors occurs at random in either the first or second division, then the frequency of segregation of sex factors in the first and second division should be equal, unless crossing over occurs. However, 25 per cent of the cell fusions of the second division should resemble those that occur when segregation takes place in the first division, that is, the fusion of the two middle cells and two end cells of the promycelium, hence the 3 : 5 ratio.

the resulting small colonies were immediately transferred to agar tubes and finally to 2 per cent malt agar in 250-cc. flasks to which enough water had been added to cover the surface in a thin film. After 2 to 3 days the flasks were shaken vigorously to bring about fragmentation of the hyphae, in the expectation that single haploid cells might become isolated in the film of water on the agar. When colonies developed, further isolations were made in the usual manner. By this method a number of haploid colonies were obtained.

It is known that a dicaryotic hypha of smut may dissociate and give rise to haploids (4, 10, 12). It seems possible that sectors in culture, which are not uncommon in *Ustilago crameri*, may in some instances originate by means of nuclear dissociation. In one case 4 different sectors were obtained from a single chlamydospore culture and these, in subsequent tests, proved to be haploid (Fig. 1). Altogether, 47 haploid lines from 8 chlamydospores were isolated from sectors.

In testing the compatibility or sexual reaction of these haploid lines on a susceptible host, no infection resulted when inoculations were made with single lines. Some of the paired combinations, however, resulted in 5 to 10 per cent infection. Different paired combinations of these lines also were observed microscopically and various stages of fusion were observed between some of them (Pl. 2, Fig. 42 to 45).

Initiation of Dicaryophase. In *Ustilago crameri*, after meiosis, each promycelial cell is usually haploid in nature but no sporidia are produced. Initiation of the dicaryophase usually results from fusion between 2 adjacent or nonadjacent cells of the same promycelium, but in certain cases fusion between promycelial cells of different chlamydospores also occurs (Pl. 1, Fig. 25 to 35 and Pl. 2, Fig. 36 to 42). The migration of the nucleus from one cell to another, that is, the initiation of dicaryophase, always takes place as soon as the special fusion structure is formed. The detailed process of migration of the nucleus can be traced quite clearly in plate 1, figures 27 to 35. After the dicaryophase is once initiated, it appears to be relatively persistent (Pl. 2, Fig. 38). However, in certain cases the newly formed dicaryophyte may revert to the haploid condition and develop quite well. Apparently, *U. crameri* is primarily homothallic but potentially heterothallic.

The dicaryophase in most smut fungi is of comparatively long duration. It generally starts shortly after meiosis and continues until caryogamy during chlamydospore formation. In *Ustilago crameri*, whether growing parasitically or saprophytically, the hyphal cells are mostly binucleate. Usually they branch and develop very rapidly after entering the host. The younger hyphal cells are so full of cytoplasm that it is hard to determine where the septa are.

As the mycelium grows, uninucleate and multinucleate cells appear more abundantly. Aberrant nuclear behavior is quite common in many different

species of the smut fungi, and even at different stages of development of a species, although the reason is not entirely clear (3, 8, 18, 22, 24).

The significance of clamp connections in the Hymenomycetes has been studied quite thoroughly, while in the smut fungi very little is known about

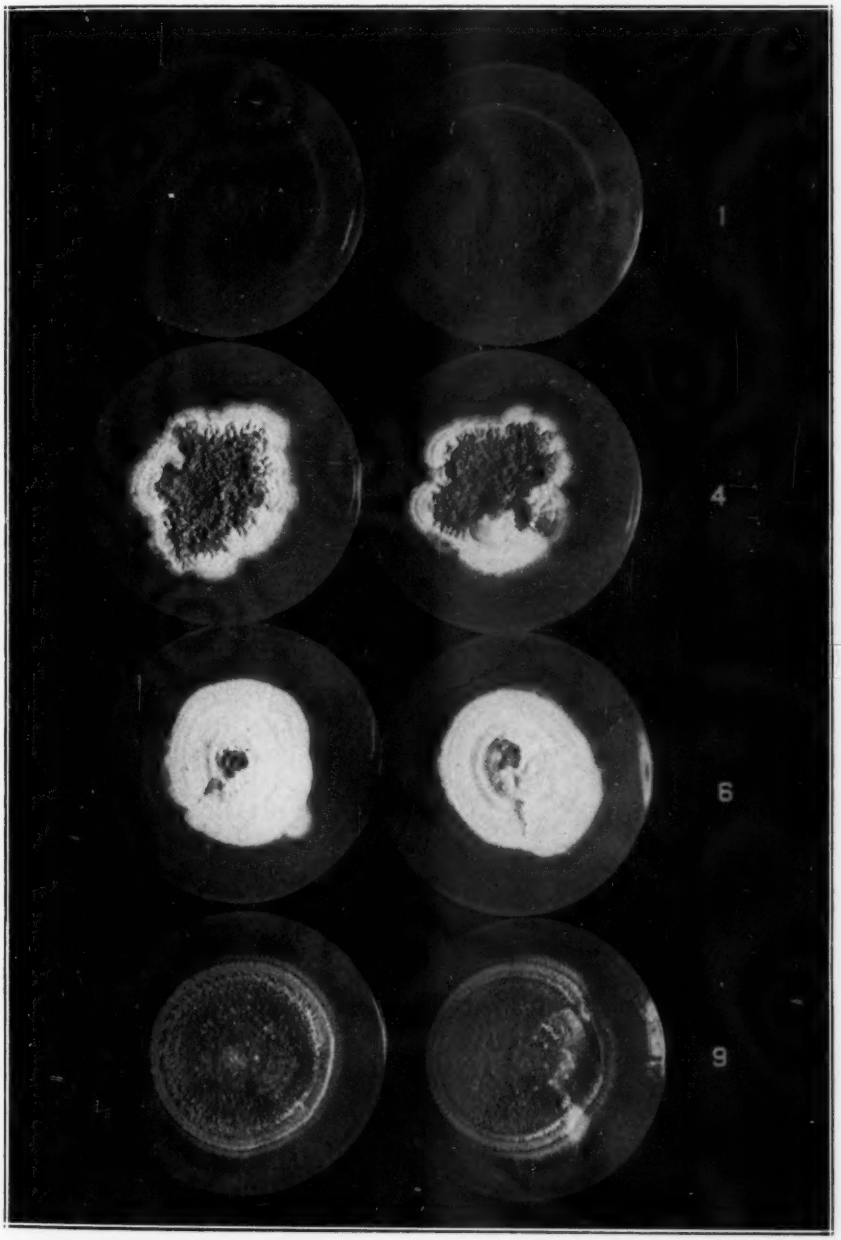

FIG. 1. Duplicate colonies of four haploid lines of chlamydospore XIII showing cultural characters that have persisted since the lines were isolated.

them. Some smuts form typical clamp connections; others form none, or only atypical ones (8, 12, 21, 22). In *Ustilago crameri*, clamp-like connections are often present on the mycelium in the host. Observations indicate that they probably are abortive hyphae.

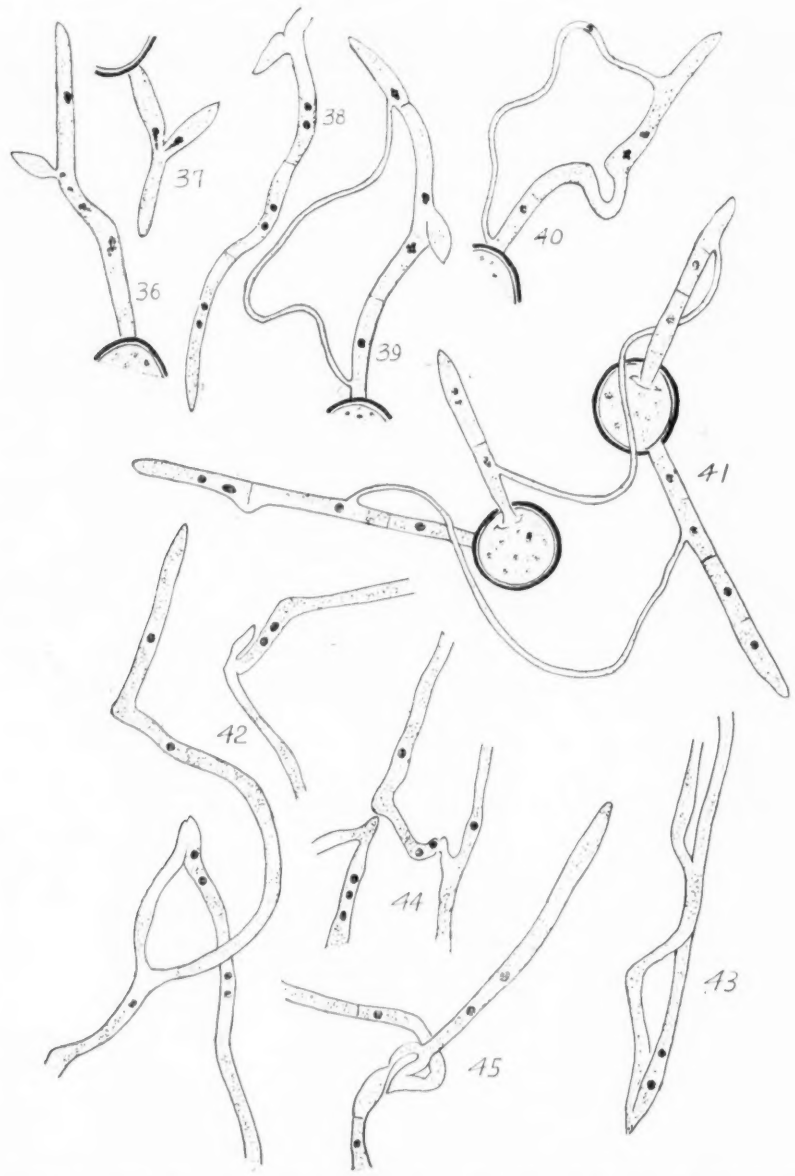

PLATE 2. Fig. 36. A sporidiumlike body. Fig. 37. Initiation of dicaryophase between two adjacent cells, showing nuclear migration. Fig. 38. A hyphal tip branching out from a promycelium, showing the binucleate condition. Figs. 39–40. Initiation of dicaryophase in non-adjacent cells, showing a nucleus in fusion tubes in fig. 40. Fig. 41. Initiation in dicaryophase between two promycelial cells of different chlamydospores. Figs. 42–45. Various stages of hyphal fusions and nuclear migrations between compatible lines. Drawn free-hand; approximately ×1,000.

Nuclear Fusion and Chlamydospore Formation. In most smut fungi the true diplophase does not begin until chlamydospore formation, and caryogamy occurs on maturation of the chlamydospores (9, 17, 18, 24). Under favorable conditions chlamydospores of *Ustilago crameri* may be formed in any part of the host plant, except the roots, within 15 days after seed germination. However, they usually are formed only in the ovary of the young spikelet of the ear. At the beginning of spore formation the hyphae, composed of binucleate cells, are both intra- and intercellular. When they reach the cells of the ovary they branch rapidly, just as in the early stages of infection. The mycelial branches, which are either uni- or binucleate, then become twisted, interwoven, and grouped into cluster-like aggregations. This is quite similar to what has been reported by various investigators for a number of other smuts (8, 9, 17, 29). The walls of the mycelial cells soon begin to gelatinize, and the cells in their sorus primordium continue to grow and swell until the process of gelatinization is complete. In the young flowers these sorus primordia may be rather numerous, sometimes coalescing and destroying the entire flower. Meanwhile, the hyphal branches appear to break up into irregular segments lying in the matrix, but the number of nuclei in such cells is not distinguishable. A little later, the gelatinized membrane disappears, and the nuclear behavior in these segments is clearer. At this time the cells change in shape and size, probably during or just before the time of nuclear fusion. Eventually, the cells enlarge, round up, form walls, and become mature chlamydospores. Apparently the spores sometimes may be formed in an intercalary manner also.

Nature of Infection. Although Kühn (see 6) and Brefeld (6) observed and recorded the time and place of infection in *Ustilago crameri*, there is no definite information on the nature of infection. This problem was investigated by the writer, particularly in relation to the nature of resistance.

In order to study the early stage of infection in millet seedlings, seeds of a susceptible variety, Kaifeng No. 142, and a resistant one, Nanking No. 31, were treated with formaldehyde in the usual manner and inoculated by dusting with a collection of chlamydospores from China. The inoculated seeds were placed between water-saturated pieces of cotton and incubated in a moist chamber at 25° C.

During germination, materials were studied in both temporary and permanent mountings. For examination of living materials, stripped epidermis or parts of the young seedlings were taken on 7 successive days, stained with safranin and gentian violet, and mounted in water. Other materials were killed, fixed, and stained at the end of the 2nd, 3rd, 5th, and 7th days after inoculation.

Materials for sectioning were fixed in either Flemming's weak solution, Nawaschin's solution, or Bouin's solution in the modifications suggested by Allen (1). To hasten penetration, the preparations were placed in a partial vacuum until the materials sank. After fixing, the materials were imbedded in paraffin and cut into sections 4 to 10 μ thick. Flemming's triple stain,

Heidenhain's iron-alum haematoxylin, Thionin and orange G., or safranin and fast green were used to stain young infected seedlings.

Twenty-four hours after the seeds had been put under favorable conditions for germination the young radicle and the plumule had emerged. Forty-eight hours after germination, most of the promycelial cells of the germinating chlamydospores on the seeds had started to fuse and infection appeared to take place during or shortly after that time.

In an effort to locate the place of infection, many inoculated seedlings, both of resistant and susceptible varieties, were examined 48 hours after germination. No difference in initial penetration into the two varieties was observed, the fungus entering the resistant variety (Nanking No. 31) as well as the susceptible one (Kaifeng No. 142). The fungus can enter any part of the seedlings, but seems to prefer the coleoptile. However, the germ tubes sometimes can penetrate the root hairs, and may grow through the root completely.

As the hypha reaches a cell wall in the coleoptile, after initial infection, it forms a spherical tip closely appressed to the cell wall. In certain cases, the part of the cell wall in contact with the hypha is stained a deep red with the triple stain, as is true also in oat smut (16). There were some indications that the hypha enters through a hole in a softened wall, but penetration by means of a fine thread or a thicker hypha also could be seen. These observations, together with the results of other investigators, led to the general conclusion that some unknown chemical changes may take place in the host-cell walls when the hyphae penetrate them.

Distribution of Mycelium in Host Tissue at Various Stages of Development

Susceptible Variety—Kaifeng No. 142. After the infection tube enters the host, it develops and branches rapidly within 24 hours. Occasionally, the hyphae in the epidermal cells are larger than those in the later stages, but this is not known to be universally true. In order to locate the mycelium at each stage of development, a series of longitudinal sections were made. In the coleoptile of a 2–4-day-old seedling the mycelium had not extended beyond the third layer of the coleoptile. Mycelium proved much more abundant in the central and upper parts of the coleoptile than in the lower part. This is possibly due more to the elongation of the coleoptile than to the selective penetration of the fungus. In 4-day-old seedlings, the new hyphae appear to be narrower than the original ones. In the meantime, the mycelium grows up and down, crosses the space between the coleoptile and the first leaf or passes directly to the meristematic regions and the vascular system and eventually enters the growing point. From now on the mycelium is either intracellular or intercellular in the vicinity of the growing point. It has been found in the adventitious roots in 21-day-old plants.

In the studies of the resistant variety Nanking No. 31, it was found that there is no difference whatever with respect to the initial penetration. Twenty-four hours after the germination of the seedlings, no branch devel-

opment of the infecting hyphae could be observed. They have been found in the second or third layer of the coleoptile in 5-day-old seedlings. Later, they disintegrate into short, isolated, intracellular segments. Of all the 19 seedlings examined, only 2 were found in which hyphae had penetrated to the meristematic region of the growing point. Further development was not followed. Since Nanking 31 is occasionally attacked by smut, the invasion by the hyphae of certain collections of *Ustilago crameri* is of course expected.

DISCUSSION

It has been known since the work of Dickinson, Hanna, Stakman, and others (11, 12, 13, 19, 23) that segregation of factors for various characters in the smuts occurred either in the first or second division of the fusion nucleus. But there was only circumstantial evidence that reduction in chromosome number had occurred, because the possibility of crossing over could not be eliminated. In the present studies it was demonstrated cytologically for the first time that reduction in chromosome number actually occurred in the first, or the second, or, occasionally, in the third division.

When chlamydospores of *Ustilago crameri* germinate, they produce a promycelium but no sporidia. The nucleus in the chlamydospore apparently is diploid. Meiosis usually occurs within the chlamydospore as it starts to germinate or may occur in the young promycelium. Eventually a 4-celled promycelium is formed, with 1 nucleus in each cell, which, on the basis of behavior and from the standpoint of the number of chromosomes, usually is haploid.

The haploid number of chromosomes is 2, as in most other smuts in which they have been seen, and the diploid number is 4. Occasionally, a diploid nucleus may be included in a promycelial cell. In such cases reduction in chromosome number occurs on the third division of the fusion nucleus. These results furnish cytological proof that some of the unexpected results that have been obtained from isolating sporidia of other smuts may be due to the fact that a promycelial cell may contain a diploid nucleus or 2 haploid nuclei instead of the usual single haploid nucleus.

As no sporidia are formed in *Ustilago crameri*, and the parasitic dicaryophase results principally from fusions between cells of the same promycelium (fusion tubes between different cells or by clamp-like connections between adjacent ones), the chances for recombination of factors for pathogenicity probably are not so great in *Ustilago crameri* as in those smuts that produce sporidia abundantly. However, fusions have been observed between cells of promycelia from different chlamydospores and this may lead to the production of new biotypes. These facts may have an important bearing on the number and constancy of physiologic forms in *Ustilago crameri*.[3]

Penetration into the host usually is accomplished by the promycelium directly, or by branches from it, which evidently are mostly dicaryotic. It appears that penetration is accomplished by mechanical pressure and possibly also by enzymatic action, as the walls of the host seem to be softened

[3] Unpublished data of the writer.

during the process of penetration. Under favorable conditions, the mycelium is well established in the host 3 days after inoculation. The early stages of infection are essentially the same for susceptible and resistant varieties. However, the mycelium rarely reaches the growing point of the resistant varieties; whereas it becomes abundant in that of susceptible varieties within a relatively short time.

SUMMARY

The mature chlamydospore of *Ustilago crameri* has a diploid nucleus, but each promycelial cell usually contains 1 haploid nucleus as a result of reduction division. In the meiotic figures, it is apparent that the chromosome number of the diploid stage is 4 and that of the haploid stage is 2.

Nuclear migration and initiation of the dicaryophase results from knee joints or fusion tubes between adjacent and nonadjacent promycelial cells and between promycelial cells of different chlamydospores. Fusions usually begin immediately after meoisis is completed.

Based on the number of chromosomes and frequency of possible fusion types between promycelial cells, it is concluded that meiosis occurs not only in the first division but frequently in the second division, and occasionally in the third.

The separation of 2 conjugate nuclei that have been associated in a single cell and the subsequent production of uninucleate hyphal cells have been observed.

Binucleate hyphal cells predominate in culture and in the host throughout the life cycle until the late stage of chlamydospore formation. The two haploid nuclei of opposite sex then fuse, and the mature chlamydospore contains only one diploid nucleus.

The process of chlamydospore formation in the host is different from that in artificial cultures. In the host, spores are formed chiefly by segmentation of the hyphal cells, while in culture they usually are formed in an intercalary manner.

The alternation of phases in general is very distinct in *Ustilago crameri;* diplophase, haplophase, and dicaryophase follow in order.

Infections were observed by dicaryotic hyphae in 2-day-old seedlings of resistant and of susceptible varieties when chlamydospores germinated on the host; the promycelia or dicaryotic hyphae derived from them function as infection hyphae and apparently penetrate by mechanical pressure, although there is some evidence of softening of the cell wall also.

In susceptible varieties the infection hyphae develop and branch rapidly and intracellularly in the coleoptile and enter the meristematic tissue as early as 4 days after seed germination, while in the resistant variety only 2 of the 19 seedlings observed were found with hyphae in the meristematic tissues.

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SOME FACTORS INHIBITING THE FRUCTIFICATION AND PRODUCTION OF THE CULTIVATED MUSHROOM, AGARICUS CAMPESTRIS L.

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INTRODUCTION

It is general knowledge among mushroom growers, particularly those engaged in growing mushrooms in underground caves or mines, that in sections lacking normal air drainage mushrooms of abnormal size and shape are formed. Where there is complete lack of air drainage, fructification ceases entirely. Although there is but little known as to the limiting factors involved, improvement in the air drainage will remedy this condition. Some light was thrown on this subject by the work of Lambert (1) who demonstrated that CO_2 in concentrations of five per cent, or more, surrounding the sporophore will cause abnormal growth and, in certain instances, death of the mushroom. No explanation is available up to the present as to the factors causing complete cessation of fructification of mushrooms under conditions where air drainage is at a minimum, and CO_2 concentrations are low. In a series of experiments an attempt was made to determine the nature of the substances responsible, or at least to find a way to remove them with methods other than increased air drainage, or circulation. These experiments, a project of the research department of Yoder Bros., were conducted in part in a limestone mine at West Winfield, Pennsylvania, Butler County Mushroom Farms, Inc., and in part at the mushroom plant of Yoder Bros. at Barberton, Ohio.

EXPERIMENTAL METHODS AND RESULTS OBTAINED

The experiments were conducted in both field and laboratory with the object of applying the results to mushroom culture under mine (cave) conditions. Their primary purpose was the removal of substances interfering with the fructification and production of mushrooms.

The "two-zone" tray system recently described by Lambert (4) was used in these experiments. In essence this system employs individual trays, or growing units, usually not exceeding 10 square feet per tray. The growing media consisted either of horse manure, or of a mixture of horse manure, corn stalks, wheat straw, and protein supplements. The horse manure received 3 to 5 turnings with an average of 17 composting days; the horse manure-organic material mixture, 8 turnings with an average of 25 composting days. Before filling the trays for the individual sub-experiments, the compost was thoroughly mixed so as to reduce the effect of compost heterogeneity on mushroom yields (2, 3). After pasteurization (sweating-out process) the trays were moved to the growing rooms, as needed for the various experiments. For field experiments the average yields of 4 single

10-sq.-ft. trays are recorded for each sub-experiment; (nature of sub-experiment not given). These small individual units were chosen for the reasons described by Lambert (3), who found that "the variance of small plots was in all cases less than the variance of entire beds. Furthermore the small plots permit a greater number of yield comparisons on the same area and also permit increased precision through replication and through the arrangement of the plots to reduce the effect of compost heterogeneity and account for the variability between beds." For laboratory experiments single square-foot units were used.

The compost was planted with a culture of a single-spore strain of the cultivated mushroom *Agaricus campestris* L., received from J. W. Sinden of the Pennsylvania State College. When the mycelium growth was complete, the trays were cased with soil for fructification and production.

Other material, as well as the methods employed, are described under each series of experiments.

Series 1. The Effect of "Locations within the Mine" on the Fructification and Production of Mushrooms

Duplicate trays of several experiments were set in different rooms of the mine in order to study the effects of "locations within the mine" on fructification and production of mushrooms. For all experiments, except 33, the mycelium was grown and fructified in the same location. In experiment 33, part of the trays, after the mycelium growth was complete, were moved to other locations for fructification.

TABLE 1.—*The effect of "locations within the mine" on mushroom production*

Mycelium grown	Mushroom produced	Number of days picked	Average yields in lb. per pounds per sq. ft.
Experiment 18. Duration: December, 1937, to July, 1938			
Mine room B	Mine room B	120	2.69
Mine room 53	Mine room 53	120	0.55
Experiment 24. Duration: January, 1938, to August, 1938			
Mine room B	Mine room B	120	1.75
Mine room 53	Mine room 53	120	0.94
Experiment 33. Duration: March, 1938, to October, 1938			
Mine room B	Mine room B	84	2.48
Mine room B	Mine room C–R	84	1.93
Mine room B	Mine room 94	84	1.81

Summary of Facts and Conclusions of Experiments Under Series 1. During the experiment it became evident that the various locations within the mine affected mushroom yields. Trays in room B consistently outyielded those in other rooms. This room, being close to the main air passage of the mine, received excellent air drainage. The air movement of the other rooms was subject to whatever air exchange was present for the

entire mine throughout the time the experiments were conducted. This air exchange varied with the prevailing atmospheric conditions outside the mine. These rooms, because of their locations within the mine, were never at optimum conditions for mushroom production.

Numerous tests for carbon monoxide and carbon dioxide were made at short intervals for the various locations in the mine. Although it was possible that the exhaust of the motor vehicles used, as well as the fumes from the workers' lamps, might have increased the monoxide content of the mine atmosphere, no trace of this gas was found in any of the samples taken. The carbon dioxide content of the air varied, but never exceeded 0.06 per cent in any sample taken. The temperature of the mine (13 to 14° C.) and the humidity (95 to 97 per cent) were constant throughout the duration of these investigations. The differences in yields obtained for the various rooms could hardly be explained by the above-mentioned factors.

It was of interest to note that, particularly at the beginning of fructification, the trays were covered with an abundance of small sporophores. Trays in room 53 not only showed the largest number of sporophores, but also strands of heavy vegetative mycelium. Quite a number of these small sporophores did not develop into mushrooms and died off. Trays in room B showed normal fructification without the heavy mycelium strand formation.

Since such a proliferous tendency to fructification is seldom found in mushroom houses, this phenomenon suggests the presence of a substance, or substances, in the mine that, in small amounts, act as a stimulant to fructification. When present in larger amounts it will not only arrest the sporophore growth, but will entirely prevent fructification.

Since the mushroom mine may be considered as a large incubator with constant humidity and temperature and limited natural air drainage, the behavior of the mushroom (or sporophore) suggests that the effect of these toxic substances is general. This assumption led to two sets of experiments that will be described as the "closed-room-effect" and the "place-effect" experiments.

The term "closed-room-effect" designates a condition in which the air circulation is kept at a minimum, allowing for the accumulation of toxic substances in such quantities as to inhibit entirely the fructification and production of the mushroom.

The term "place-effect" designates the condition of growing mushrooms in both mine and mushroom house and measuring the difference in yields in order to establish a relative depressing effect of the mine at any given time.

Series 2. The "Place Effect" on the Fructification of Mushrooms

From the numerous experiments in this series conducted from 1938 to 1943, inclusive, only 4 will be presented here to demonstrate the fluctuation of the mine atmosphere and its effect on mushroom fructification and pro-

duction as compared with house atmosphere. In these experiments notes also were taken on the development of mycelium under these conditions. For comparative tests identical sets of trays were grown in the mine and the mushroom house. The mushroom house located at Barberton, Ohio, will be designated as "house," and the limestone mine (cave) located at West Winfield, Pa., as "mine" (Table 2).

After completion of the growth of the mycelium, halves of either set of trays were interchanged in order to study the "place effect" on the fructification and production of mushrooms.

TABLE 2.—*The place effect of house and mine culture on the production of mushrooms*

Mycelium grown	Mushrooms produced	Number of days picked	Pounds per sq. ft. sub-experiment				
Experiment R–9B. Duration: Oct., 1938, to May, 1939							
			1	2	3	4	5
House	House	94	1.41	1.57	1.76	1.66	1.88
House	Mine	94	1.45	1.71	1.92	1.83	2.12
Experiment EE. Duration: Oct., 1940, to July, 1941							
			EE–1	EE–2	EE–3	EE–4	EE–5
Mine	Mine	106	1.30	0.80	1.12	1.51	1.67
Mine	House	61	1.71	1.29	1.65	2.32	2.38
House	House	128	2.42	1.62	2.40	2.91	2.55
House	Mine	125	1.87	1.51	2.03	2.42	2.17
Experiment A–B. Duration: Oct., 1941, to May, 1942							
			6	7	13	15	16
House	House	80	1.87	2.29	2.11	2.33	2.16
House	Mine	80	1.49	1.84	1.51	1.72	1.51
Experiment 1A, 1B, 3A. Duration: Oct., 1942, to March, 1943							
			1A	1B	3A		
House	House	94	2.01	2.16	1.81		
House	Mine	84	1.86	2.21	1.85		
Mine	Mine	67	1.88	2.07	1.97		

Summary of Facts and Conclusion of Experiments Under Series 2. Mycelium, grown under mine or house conditions, was excellent. There was no visible "place effect" on its development.

Conditions under which fructification and production of mushrooms took place varied. Since the trays of experiment R–9B were placed directly in the main air passages of the mine, the constant natural air flow present was sufficient to prevent accumulation of substances interfering with the fructification and production of the mushrooms. In this experiment no place effect of the mine could be observed. However, trays placed in other sec-

tions of the mine, but not included in this experiment, showed a distinct place effect, that is, lower yields resulted.

Shortly before experiment 1A, 1B, and 3A was started, an artificial ventilation system was installed in the mine. Inasmuch as the mine yields obtained in this experiment equalled house yields, it may be concluded that the artificial ventilation of the mine was sufficient to remove any harmful gases present.

The results of the two other experiments (EE and A–B) reflect the unfavorable atmospheric conditions of the mine. During the experimental periods there was little actual air movement in the mine, and the accumulation of toxic substances must have been considerable. Increased sporophore formation, dying of small mushrooms, small-sized mushrooms and lower production yields resulted as compared with those produced under house conditions during the same period.

These experiments have failed to show why mine mushrooms are smaller on the average than house mushrooms. The causes for this phenomenon are not necessarily associated with production yields. Production in the mine was equal to house production, but the mine mushrooms were smaller in size than the house mushrooms (Table 2, Exp. R–9B and 1A, 1B, 3A).

Series 3. The Closed-room Effect on the Fructification and Production of Mushrooms

Experiments in Series 1 and 2 demonstrated that mine rooms having but little air movement exerted a harmful effect on the fructification and production of mushrooms. The question naturally arises as to what would happen if the air circulation in the mine room was cut off entirely.

The dead ends of two mine rooms were sealed off with building paper from the remainder of the rooms. No attempt was made to have them absolutely air-tight. The sealed-off sections were labeled "inside" and the remaining rooms were marked "outside," and had access to the normal air drainage of the mine. Trays with previously prepared compost were set up in each section, and the mycelium was grown both "inside" and "out-

TABLE 3.—*The effect of "inside" and "outside" of mine rooms on fructification and production of mushrooms*

(Duration of the experiment: Dec., 1938, to Aug., 1939)[a]

Mycelium grown	Mushrooms produced	Lb. per sq. ft.
Inside room 54	Inside room 54	0.00
Inside room 54	Outside room 54	0.77
Outside room 54	Inside room 54	0.00
Outside room 54	Outside room 54	1.32
Inside room D	Inside room D	0.00
Inside room D	Outside room D	0.77
Outside room D	Inside room D	0.02
Outside room D	Outside room D	1.48

[a] Dr. E. F. Hopkins of the Yoder Bros. Research Department was in charge of this experiment at West Winfield, Pa.

side." The other halves of either section of the rooms remained where they were grown.

Summary of Facts and Conclusion of Experiment under Series 3. From its appearance the mycelium grown both "inside" and "outside" appeared excellent.

Fructification, however, took place only in the "outside" sections of the rooms; and this occurred irrespective of where the mycelium was grown. There was one exception, however. Mycelium grown "outside" room D and then moved "inside" the same room produced some mushrooms. The reason for this apparent contradiction of the closed-room effect on fructification was found in later studies given under Series 4. Here it was demonstrated that when sporophore formation took place before the trays were exposed to the closed-room effect some of the mushrooms developed. This undoubtedly took place with the mycelium grown "outside" room D, since, as has been frequently observed, mycelium grown under mine conditions will fructify, even in absence of casing soil. Undoubtedly some of the mycelium in this group of trays fructified before it was cased and placed "inside." Hence the mushroom yields recorded.

Mycelium grown "inside" and then moved "outside" for fructification produced only half as much as that grown and left "outside" for production. This would indicate that mycelium grown under closed-room conditions (inside) has a lower production capacity.

The mine with its limited natural air flow can be considered a semi-closed room, and mycelium grown under such conditions should have a production capacity inferior to that grown under house conditions. This actually has been demonstrated. As will be recalled (Experiment EE, Table 2) mycelium grown under mine conditions resulted in inferior yields when compared with that from house conditions. When there is a positive and continuous air movement (artificial ventilation) mycelium grown under mine conditions will produce equally as well as that grown under house conditions (experiment 1A, 1B, 3A).

Series 4. The Fructification of Mushrooms under Controlled Atmospheric Conditions

In the preceding experiments it has been shown, that in mines (caves) lacking air drainage or exchange, mushroom fructification and production will be affected. Questions arise as to the nature of the substances responsible and the possibility of removing them.

It is general knowledge that waste products formed in the metabolic processes of fungi exert a harmful effect on the producing organism. The term staling products has been applied because the nature of these products is not fully understood. Similar substances are produced by the microflora responsible for the decomposition (conditioning) of the organic materials used in the preparation of the mushroom compost. An accumulation of these products continues in this medium, coming in part from the microflora

present, and in part from the cultivated mushroom itself. In commercial mushroom culture it is impossible to remove these substances from the growing medium either by washing or leaching. Additions of chemicals such as copper sulphate to the growing medium or the improvement of its oxidation-reduction system through the use of manganese sulphate and ferric sulphate resulted in increased yields (unpublished data). Aside from those that combined with the medium itself, there are some that, due to their volatile nature, are released into the surrounding atmosphere. These only will be considered in this paper.

It has been shown that air polluted with these volatile substances will be detrimental to the fructification and production of mushrooms. Under normal growing conditions, such as prevail in mushroom houses, these substances are readily removed and carried off with an exchange of air. Furthermore, the fluctuation of temperature (and humidity) in the houses and its effect on the periodic drying of the casing soil and the culture medium, will greatly facilitate the removal of these gases.

Due to the constant temperature and humidity conditions in the mushroom mine, it can be assumed that the rate of liberation of these substances from the growing media is slow. Such an accumulation in the medium will affect fructification and production of mushrooms. If present in small amounts, such substances may stimulate vegetative growth. It has been observed generally that mycelium grown under mine conditions is more profuse than that grown under house conditions. The proliferous sporophore formation may also fall under such a stimulant effect. It is an established fact that such a proliferous sporophore formation usually results in a crop of small mushrooms, particularly in mines. High accumulations of these substances in the culture medium, as well as in the surrounding atmosphere, will stop fructification entirely. The term "closed-room effect" has been applied to such conditions (Series 2).

The removal of these substances by artificial air exchange, a problem of mechanical nature, has not been considered in these studies. Due to the volatile nature of these substances the following experiments were designed to remove them by washing the air.

EXPERIMENTAL METHOD AND PROCEDURE

To simulate cave, or closed-room conditions, special chambers for growing mushrooms were built. The frames were made of wood and painted with du Pont primer. The sides were of glass. One end of the chamber was removable, and after it was loaded with the mushroom trays, it was sealed with putty. There was an outlet on each end for air-tube connections. These chambers had a capacity of 12 cu. ft. each, and were set in a large room in which the average temperature was 14° C. Small air pumps, 2 cu. ft. capacity per minute, were used for circulating the air in the mushroom chambers. The air was pumped through the various air-washing equipment periodically. Water traps were installed between the solutions

used, except in the sulphuric acid experiment, where the desired relative humidity in the culture chambers was maintained. The following materials for washing the air were used: 1. Alkaline potassium permanganate solution 3 lb. of sodium hydroxide plus 2 ounces of potassium permanganate per 5 gal. of water. 2. Dorex Cannister containing activated charcoal, obtained from the W. B. Connor Engineering Co., New York, N. Y. 3. Mineral oil (commercial grade). 4. Sulphuric acid (commercial grade).

The mycelium for these experiments was grown in special sq. ft. trays. After completion of the growth, the trays were cased with soil, watered, and placed in the various chambers. After loading the chambers, the outlets were connected with the air-washing equipment by means of rubber tubing.

Duration of the experiment: August, 1942, to March, 1943.

Experiment A

In this set the mycelium was grown under house conditions. After the trays were cased with soil they were placed in the chambers. The air in chambers 1 and 2 was washed periodically at 6-hour intervals, moving 40 cu. ft. of air through the washing units. Chamber 3 did not receive any washing.

Chamber 1. Direction of air flow: mushroom chamber—airpump—water trap—mineral oil—water trap—alkaline potassium permanganate solution—water trap—return to mushroom chamber. *Results.* Sporophores formed after 17 days of washing. The development of the fruit bodies was normal.

Chamber 2. Direction of air flow: mushroom chamber—airpump—Dorex Cannister—return to mushroom chamber. *Results.* Sporophores formed after 19 days of washing. The development of the fruit bodies was normal.

Chamber 3. No washing of the air. *Results.* A heavy surface growth of the mycelium appeared on top of the casing soil. No sporophore formation occurred.

Experiment B

As in experiment A, mycelium that had completed its growth was used in this set. Here, an attempt was made to differentiate between the ability of mineral oil and that of alkaline potassium permanganate solution to remove the substances. The same period for washing the air was maintained as in experiment A.

Chamber 1. Direction of air flow: mushroom chamber—airpump—water trap—alkaline potassium permanganate solution—water trap—return to mushroom chamber. *Results.* Sporophores formed after 16 days of washing. The development of the fruit bodies was normal.

Chamber 2. Direction of air flow: mushroom chamber—airpump—water trap—mineral oil—water trap—return to mushroom chamber. *Results.* Sporophores formed after 16 days of washing. The developing fruit bodies showed the typical CO_2 effect, as described by Lambert (1).

The stipe was elongated and the pileus was retarded in its development, giving the mushroom an out-of-proportion appearance.

Chamber 3. No washing of the air. *Results.* Similar to those as given under chamber 3, experiment A.

Experiment C

In this set an attempt was made to determine firstly (tray *a*), whether trays in the process of fructification and production would continue if placed under closed-room conditions; secondly (tray *b*), whether cased mycelium exposed to closed-room conditions for a period of 28 days could be made to fructify and produce mushrooms; thirdly (tray *c*), the use of outside-grown mycelium for comparison.

After loading the trays in the chamber, the air in chambers 1 and 2 was washed for the same interval as given under experiments A and B.

Chamber 1 and 2 (duplicates). Direction of air flow: mushroom chamber—airpump—water trap—alkaline potassium permanganate solution—water trap—return to mushroom chamber.

Tray a. Results. Sporophores continued to appear. Fruit bodies present before trays were put into chamber continued to develop normally.

Tray b. Results. Sporophores developed after 26 days of washing. The total number formed was somewhat less than those forming on tray *c*. The development of the fruit bodies was normal.

Tray c. Results. Sporophores formed after 16 days of washing. The development of the fruit bodies was normal.

Chamber 3. No washing of the air.

Tray a. Results. No further sporophores formed. Some of the fruit bodies present developed into mushrooms of gigantic proportions. The length of stipe was about 2 to 3 times that of outside-grown mushrooms. It was swollen at the base and of dumbbell shape. The pileus was abnormally large, misshapen, and not so symmetrical as that of a normal mushroom. Some fruit bodies had a small pileus resembling those formed under conditions having a high CO_2 concentration of atmosphere. The rate of development of these fruit bodies was very rapid, and at maturity they soon collapsed. Other fruit bodies remained "seated." They bulged, onion-like, at the base.

Tray b and c. Results. No sporophore formation. There was a heavy growth of mycelium on top of the casing soil.

Experiment D

In this set an attempt was made to determine whether the relative humidity of the air surrounding the mushroom trays affects sporophore formation and the development of the fruit bodies. Sulphuric acid served as the humidity-control agent. The concentrations of the acid were determined by specific-gravity measurements (5, 6). The alkaline potassium permanganate solution was employed for removal of the volatile gases. The air in

the chambers was washed at 2-hour intervals over a period of 28 days. The sulphuric acid was replaced every 4 days through the period of the experiment. Mycelium grown under outside conditions was used after completion of its growth.

Chamber 1. Direction of air flow: mushroom chamber—airpump—water trap—alkaline potassium permanganate solution—water trap—sulphuric acid at a concentration to maintain an average humidity of 35 per cent—return to mushroom chamber. *Results.* Sporophores formed after 18 days of washing. The developing fruit bodies were normal in shape, except for their size. It was observed that these mushrooms were more compact and had a shorter stipe and smaller pileus than mushrooms grown under outside conditions.

Chamber 2. Direction of air flow: mushroom chamber—airpump—water trap—alkaline potassium permanganate solution—water trap—sulphuric acid at a concentration to maintain an average humidity of 88 per cent—return to mushroom chamber. *Results.* Sporophores formed after washing 19 days. The developing fruit bodies were of normal shape and size.

Summary of Facts and Conclusions of Experiments under Series 4. Trays placed in air-tight chambers receiving no air washing failed to fructify. Conditions in these chambers did not interfere with the growth of the mycelium. A heavy surface growth on top of the casing soil was present on all of the trays exposed to these conditions.

Trays in the process of production placed in these chambers exhibited an interesting picture. No new sporophore formation took place. Fruit bodies present before the trays were placed in the chambers continued to develop. Some of them grew into mushrooms of abnormal shape and size, others remained "seated," bulging, onion-like, only at the base of the stipe. The large fruit bodies formed collapsed soon after reaching maturity and disintegrated. Their keeping quality was poor in comparison with outside-grown mushrooms. Sister trays, placed in chambers from which the toxic substances were removed, continued to fructify and develop normal mushrooms.

Removal of the toxic substances inhibiting fructification was accomplished with either mineral oil, alkaline potassium permanganate solution, or activated charcoal. Since mineral oil did not remove the CO_2, an accumulation of this gas in the chamber resulted in malformed mushrooms, as described by Lambert (1). The fact that these substances can be removed with mineral oil suggests that their nature may be that of unsaturated hydrocarbons, readily soluble in a highly purified hydrocarbon. Solutions of alkaline permanganate removed the toxic substances by oxidation. This implies that these toxic substances may be of the nature of nonsaturated hydrocarbons, which are readily oxidized.

The reason for the common practice of supplying air to growing mushrooms can be explained on this basis. Positive air movement insures a bet-

ter oxygen supply, particularly in regions where the mycelium grows. Under mushroom-house conditions, this is easily accomplished. The frequent watering and drying of the mushroom media will aid this process; hence, normal mushroom yields in houses. Under mine conditions, due to constant temperature and humidity, there is a minimum of air movement, which, in part, explains the lower mushroom yields obtained under these conditions.

Activated charcoal, the principal ingredient of the Dorex Cannister, was also effective in removing the toxic substances, together with the CO_2 present.

Whether the humidity was maintained at either 35 or 88 per cent was immaterial for sporophore formation in these experiments. Low humidity, however, restricted the development of the fruit body, giving mushrooms of normal shape but smaller than those formed in higher humidity.

It was of interest to note that mycelium previously exposed to closed-room conditions over a period of 28 days after casing could be made to fructify by washing the air. The mycelium, however, needed a longer time for sporophore formation than normally cased mycelium. Whether this delay in fructification was due to an exposure of the mycelium to closed-room conditions after casing, or to a weakening of the mycelium by prolonged respiration has not been determined.

The very fact that substances belonging to the unsaturated hydrocarbons are effective in inhibiting and preventing fructification of the cultivated mushroom *Agaricus campestris* without interfering with the growth of the mycelium proper, as shown in this paper, suggests that: Substances of the same nature could be used effectively in the control of some plant diseases caused by fungi. Preventing the fungus from fructifying eliminates dissemination of spores and the disease will be restricted to its primary infection centers, even though the mycelium may continue to grow unchecked vegetatively as these substances may not have fungicidal value in the accepted terms.

SUMMARY

Conditions affecting mushroom fructification and production have been investigated.

It was found that wherever mushrooms are grown, substances of volatile nature accumulate and become detrimental to their growth and fructification.

Under mine conditions certain rooms tend to accumulate these substances more readily than others, and the term "locations within the mine" has been applied to this phenomenon.

In mushroom houses high yields are produced as a result of constant mechanical ventilation, whereas in mines where the air exchange is dependent upon natural ventilation, yields are lower. The term place effect describes these conditions. Improving the ventilation in the mine will raise yields to the production level of the mushroom house.

When these substances were permitted to accumulate, fructification

ceased entirely. This was accomplished by sealing up mine rooms or experimental chambers (closed-room effect).

The effect of these substances on mushrooms varied. Mycelium grown where these substances were allowed to accumulate had a lower production capacity than that grown where they were removed. It was impossible, visibly, to distinguish between mycelium grown under either conditions. Under mine conditions where there was but a partial removal of these substances, the effect on fruit bodies was observed in various stages ranging from increased fructification (sporophore formation) to complete cessation of fructification. Sporophores exposed to conditions allowing for an accumulation of these substances either developed into fruit bodies of gigantic size and abnormal shape, or they did not elongate at all and grew only at the base of the stipe, assuming an onion-like appearance.

Removal of these substances has been accomplished by washing the atmosphere. Alkaline potassium permanganate solutions, mineral oil, and activated charcoal proved to be effective agents for this purpose.

The nature of these substances is unknown. However, the chemicals successfully used for their removal suggest that they belong to the class of nonsaturated hydrocarbons.

Their origin has not been determined, but it is assumed that they are products of the metabolism of the mushrooms themselves, or of the microflora present in the growing media.

Humidity control of the atmosphere surrounding the trays did not affect sporophore formation.

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THE FUNCTION OF LIME AND HOST LEAVES IN THE ACTION OF BORDEAUX MIXTURE

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The importance of lime in Bordeaux mixture may be emphasized by the fact that, although the fungicidal value of copper sulphate was reported by Prévost in 1807 (10), it was not until lime was added to the bluestone solution that a protective copper fungicide suitable to foliage was developed by Millardet, and reported in detail in 1885 (9). Millardet found that 0.01 per cent lime or 0.000025 per cent bluestone would inhibit the germination of the sporangia of *Plasmopara viticola,* but the spray he recommended for field control contained 1190 times the above content of lime and 238,000 times the above content of copper, indicating that Millardet was possibly aware of some great apparent discrepancy between *in vitro* and *in vivo* values of the fungicidal value of copper. According to Millardet (9), "The lime seems to me, then, to play a triple role in the mixture. At the moment of spraying it acts as an energetic mordant, which fixes the disinfecting drop on the leaf and establishes its close adherence. For several days, it is capable of killing the conidia and zoospores by its causticity. Finally, when it has been transformed into carbonate, it serves for the preservation of the store of copper oxide." It is this last function of lime listed by Millardet, but which might be stated differently, that the writer considers most important, and that will be treated in this paper. Another important aspect of the function of lime, not considered by Millardet, and not treated in this paper, is the injurious effect of lime on the host, as discussed by Horsfall and Suit (6).

Several investigators have indicated differences between the apparent *in vitro* and *in vivo* toxicity of copper sprays. Marsh (8) reported that conidia of *Venturia inaequalis* did not germinate on slides sprayed with copper cyanide, but germinated to the extent of 39 per cent on leaves to which the same dosage of copper cyanide had been applied. Howard (7) showed that several samples of Bordeaux were more toxic to *Alternaria solani* conidia on glass slides than on potato leaves. Something in the leaves apparently reduced the toxicity of the copper. It has been shown by Clark (2) and confirmed by the writer (12) that asparagin, a normal constituent of leaves, may greatly reduce the toxicity of copper sulphate or Bordeaux in *in vitro* tests. The literature on antagonism in relation to copper is quite extensive, and will not be reviewed here.

METHODS

The dosage-response technique of Wilcoxon and McCallan (11) was used in this study, and the fungicidal value of lime alone, of bluestone alone, and of mixtures of bluestone and lime were measured throughout a series of

dosages on glass and leaf surfaces. The dosage increment (ratio of concentration of successive dosages) of 3 generally used in this study, is more than that used by Wilcoxon and McCallan and is probably too great for careful studies of such aspects as changes in slope of the dosage response curve, but was considered adequate for purposes of this study. Dosages were chosen to determine principally the LD 50, LD 95, and intermediate values, to the considerable neglect of values below LD 50. The straight line best fitting the points in the region of LD 50 to LD 95 is taken as the dosage response curve. Many experiments gave approximately straight line curves, but a more detailed study might show these curves to be other than straight lines.

Bordeaux mixture was prepared by adding equal amounts of 10 per cent bluestone ($CuSO_4 \cdot 5H_2O$) and 10 per cent lime (CaO) to the required amount of water, unless otherwise mentioned. The stated concentrations of Bordeaux are the concentrations of bluestone in this equal lime-Bordeaux mixture.

Bean rust, cucumber downy mildew, and powdery mildew of bean were the diseases studied in the greenhouse and laboratory experiments to which this report is confined. Bean rust (*Uromyces phaseoli* (Pers.) Wint.) was grown on the primary true leaves of potted Pinto beans. To secure spores, infected leaves were sprayed with tap water by means of an atomizer, and the suspension of urediospores secured in this way was sprayed immediately onto the surfaces bearing the dried test spray deposit—Syracuse watch glasses for germination tests, leaf surfaces for protection tests. The concentration of spores was not determined, but was such as to give from 2 to 25 spores per sq. mm. in germination tests and 40 to 125 pustules per sq. cm. on control leaves in protection tests. Inoculated plants were held overnight in a moist chamber and then left on the greenhouse bench until infection was measured by counting the uredinial pustules.

Cucumber downy mildew (*Pseudoperonospora cubensis* (B. and C.) Rostew.) was grown on the secondary leaves of potted cucumber plants, variety Long Green. To secure sporangia, leaves that had been infected 5 to 10 days were incubated overnight in moist chambers, and the sporangia formed were washed off to form a water suspension, used to inoculate sprayed and control plants in protection tests and to seed onto spray deposits on glass. The spore dosage was such as to give about 5 sporangia per sq. mm. germination tests and about 20 downy mildew lesions per sq. in. on control leaves in protection tests.

In protection tests on bean and cucumber foliage a range of dosages was secured by using sprays of different fungicide content and spraying to the run-off stage with a compressed-air atomizer. The initial wet deposit per sq. dm. of leaf surface secured in this way without a spreader was variable, but averaged about 1 g. for the lower surface of bean leaves, 0.8 g. for the upper surface of bean leaves, 2 g. for the lower surface of cucumber leaves and 1.5 g. for the upper surface of cucumber leaves. Sprays without supplements usually gave fair coverage on beans but poor on cucumbers. When

0.05 per cent glyceryl phthalic alkyd resin was added as a spreader to the sprays the deposit per sq. dm. was about 0.6 g. for the upper or lower surfaces of bean leaves, 1 g. for the lower surface of cucumber leaves and 0.7 g. for the upper surface of cucumber leaves. The amount of fungicide per unit area was calculated from the above figures on wet deposit and from the known fungicide content of the spray. For infection counts in protection tests the number of rust pustules in 3 to 8 (depending on variability and amount of infection) sq. cm. areas was counted for each treatment, and these values were converted to per cent reduction in number of rust pustules (= per cent control) by expressing the difference in the number of pustules per unit area for treated and control (= check) leaves as a percentage of the number of pustules on the control leaves. For infection counts of cucumber downy mildew the number of lesions on 2 to 4 sq.-in. areas was counted for each treatment and the per cent of control was determined in the same way as for bean rust.

Powdery mildew of the bean (*Erysiphe polygoni* DC.), grown on the primary leaves of Pinto beans, was employed in a study of the eradicant action of fungicides. Bean plants were inoculated by spraying the leaves on both surfaces with a suspension of conidia secured from infected plants. At 4 to 6 days after inoculation, when infection was just visible, the inoculated plants were sprayed with the test spray containing 0.05 per cent phthalic glyceryl alkyd resin as a wetting agent in addition to the test dosages of bluestone or lime. The wetting agent was added because it increased the uniformity of the results and increased the effectiveness of most sprays, but had very little effect when used alone at the low impact pressures used in these tests. The choice of 4 to 6 days as the interval from inoculation to the use of eradicant sprays is arbitrary but it is important to bear in mind that the resistance of powdery mildew colonies to killing by eradicant sprays increases with the age of the colonies (data not presented here). At about 5 days after spraying (9 to 11 days after inoculation) the amount of mildew was estimated on an arbitrary scale of 0 to 10 in which 0 indicated no mildew present and 10 that the leaf surface was entirely covered. The upper and lower leaf surfaces were rated independently. Per cent control was calculated by expressing the difference in rating between treated and control leaves as a percentage of the rating for the control.

In studies of the effect of copper sulphate and lime on germination, 3 cc. of the test spray was added with a pipette to Syracuse watch glasses of 51 mm. diameter and allowed to dry. This gave a uniform deposit of fungicide of known dosage onto which the spore suspension was later sprayed. The cultures were incubated at 16° C. in the dark for 24 hours and the germination of 100 spores per dish for each test was determined. The presence of a germ tube was the criterion of germination in the case of bean-rust urediospores, and the absence of contents and papilla was the criterion of germination for the sporangia of cucumber mildew. The heavy deposit of lime required to inhibit germination of bean-rust urediospores and downy-mildew

sporangia, and the heavy deposit of Bordeaux required to inhibit germination of bean-rust urediospores made observations of germination in these *in vitro* cultures very difficult and the results rather inaccurate.

PHYTOCIDAL ACTION OF SPRAY MIXTURES

In order to evaluate properly the effect of a fungicide in controlling a fungus disease it is desirable to know the effect of the fungicide on the host in the absence of the disease. As is well known, bluestone is more toxic to foliage than lime or Bordeaux mixture at similar concentrations. Results of one comparative test on beans and cucumbers are given in figure 1. In this test plants with secondary leaves were sprayed with the test mixtures on September 2, 1942, and the living tissues of the leaves were weighed on September 7. Bluestone at 0.1 per cent caused about 5 per cent injury and

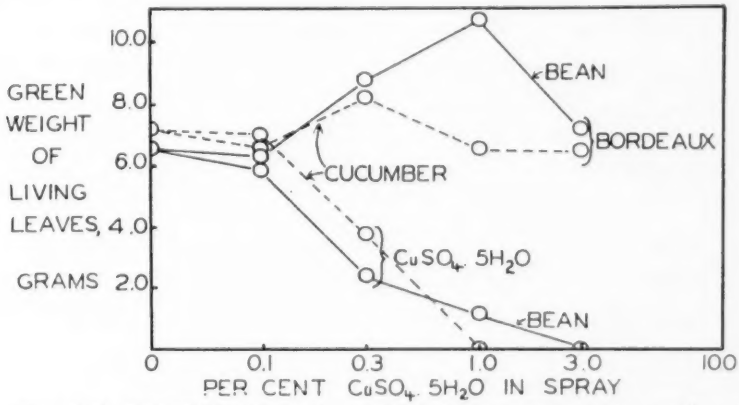

FIG. 1. The phytocidal effect of bluestone and Bordeaux on bean and cucumber foliage.

3 per cent bluestone caused complete killing of the bean and cucumber foliage, while 3 per cent Bordeaux did not cause marked injury. Lime alone was not used in this test, but in other tests lime has caused no apparent injury at 1 per cent.

PROTECTIVE ACTION ON LEAVES AND TOXICITY ON GLASS

Results of two tests of the protective action of bluestone, lime, and Bordeaux for bean rust are summarized graphically in figure 2. Here the concentration of the spray applied to the leaves is plotted against per cent reduction in number of rust pustules (= per cent control). These results are representative of the variation between tests, and of the deviation from straight lines of the dosage response curves plotted in this manner. In order to render results of this type more closely comparable to those on glass surfaces, all results were converted to a dosage-per-unit-area basis. Average LD 50 and LD 95 values for bean rust of all comparative tests of the effect of bluestone, lime, and Bordeaux on the inhibition of germination on glass and the protection from infection on leaves are presented in table 1.

Several important findings are indicated from the data of table 1. To obtain 95 per cent inhibition of germination of bean-rust urediospores on glass required more than 100 times as much copper in the form of Bordeaux as in that of bluestone, indicating that lime decreased the fungicidal action of the copper on glass surfaces. On the other hand, to obtain 95 per cent control on leaves required more than 10 times as much copper in the form of bluestone as in the form of Bordeaux, indicating that lime enhanced the fungicidal value of the copper on leaves. To obtain 95 per cent reduction in germination on glass required 350 times as much Bordeaux mixture as to obtain 95 per cent control on leaves, further emphasizing the important difference between glass and leaves as a substrate for the evaluation of fungicidal action.

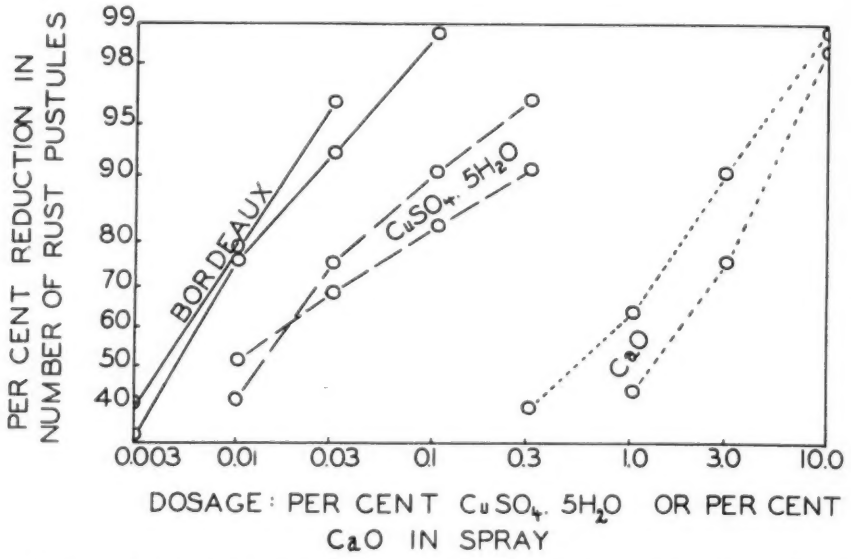

FIG. 2. Relation between spray dosage and protection (reduction in number of uredinial pustules) from bean rust.

TABLE 1.—*Comparative toxicity and protective value of lime, bluestone, and Bordeaux mixture for bean rust*

Fungicide	Germination on glass.[a] Dosage per square decimeter for		Protection on plants.[b] Dosage per square decimeter for	
	50 per cent reduction in germination	95 per cent reduction in germination	50 per cent reduction in number of rust pustules	95 per cent reduction in number of rust pustules
CaO	60 mg. CaO	300 mg. CaO	28 mg. CaO	124 mg. CaO
$CuSO_4 \cdot 5H_2O$	0.113 mg. Cu	0.60 mg. Cu	0.062 mg. Cu	2.1 mg. Cu
Bordeaux	15 mg. Cu	64 mg. Cu	0.022 mg. Cu	0.18 mg. Cu

[a] Average of 6 tests for CaO, 9 for $CuSO_4 \cdot 5H_2O$, and 11 for Bordeaux.
[b] Average of 3 tests for CaO, 8 for $CuSO_4 \cdot 5H_2O$, and 8 for Bordeaux.

A source of error not allowed for in these tests was the greater spore dosage per unit area in the toxicity tests on glass than in the protective tests

on leaves. The spore dosage of the inoculum on leaves was not determined, and, since infection results were recorded as pustules per sq. cm., the writer does not know with certainty how spore-dosage data could be made use of here, even though they were known.

Studies of the comparative toxicity and protective values of lime, bluestone, and Bordeaux for cucumber downy mildew are presented in table 2. These results differ from those for bean rust in several important respects. The critical toxicity values for bluestone and Bordeaux are similar, and both are much less than for bean rust, showing that copper is much more toxic to the cucumber downy-mildew organism than to the bean-rust fungus, and possibly that the downy-mildew sporangia are able to release copper from Bordeaux films on glass, while bean-rust urediospores are not, or have only weak copper-dissolving powers. At the LD 50 level of fungicidal action no marked differences are apparent between the results for cucumber downy mildew on glass and on leaves, while at the LD 95 level both bluestone and Bordeaux are much more effective per unit of copper on glass than on leaves. Because of the poor spreading properties of the sprays on leaves it is difficult to appraise these differences. Another important result shown in table 2, for which no explanation is offered, is the much greater increase in control resulting from adding spreader to the Bordeaux than in adding the spreader to the lime or bluestone.

TABLE 2.—*Comparative toxicity and protective value of lime, bluestone, and Bordeaux mixture for cucumber downy mildew*

Fungicide	Germination on glass. Dosage per square decimeter for		Protection on plants. Dosage per square decimeter for	
	50 per cent reduction in germination	95 per cent reduction in germination	50 per cent reduction in number of lesions on leaves	95 per cent reduction in number of lesions on leaves
CaO	40 mg. CaO	113 mg. CaO	21 mg. CaO	122 mg. CaO
CaO + 0.05 per cent spreader[a]			5.2 mg. CaO	33 mg. CaO
$CuSO_4 \cdot 5H_2O$	0.0056 mg. Cu	0.049 mg. Cu	0.008 mg. Cu	2.2 mg. Cu
$CuSO_4 \cdot 5H_2O$ + 0.05 per cent spreader[a]			0.003 mg. Cu	0.45 mg. Cu
Bordeaux	0.0074 mg. Cu	0.060 mg. Cu	0.012 mg. Cu	1.7 mg. Cu
Bordeaux + 0.05 per cent spreader[a]			0.0009 mg. Cu	0.037 mg. Cu

[a] 0.05 per cent phthalic glyceryl alkyd resin, used as a spreader in these tests on foliage, gave 45 per cent protection (average of 3 tests) when used alone.

RELATION OF COPPER ABSORPTION BY LEAVES TO FUNGICIDAL PROTECTION

The injury from bluestone applied to leaves (Fig. 1) and the greater control of bean rust by Bordeaux than by bluestone (Fig. 2) suggested the absorption of copper by bean leaves in large amounts from bluestone but not from Bordeaux. To measure this copper absorption indirectly, and determine its effect on the protective value of copper, one set of bean leaves was

sprayed with 0.1 per cent bluestone, and opposite leaves of the same plants were sprayed with 0.1 per cent Bordeaux. These leaves were used for copper analysis or were inoculated with bean rust immediately or after being held for 4 hours in a moist chamber. The sprayed leaves on plants held in the moist chamber for 4 hours were still wet on removal. To determine copper the leaves were immersed in 0.5 per cent nitric acid and agitated occasionally for 20 minutes and then removed. It was hoped in this way to remove the copper from the surface of the leaves, but not the copper within the leaves. To this nitric acid extract 0.01 per cent sodium diethyl dithiocarbonate was added (1), and the copper was determined by comparison with standards of known copper content in a photoelectric colorimeter.

The results presented in table 3 indicate that over half the copper in bluestone spray was absorbed by the leaf in 4 hours if the leaf remained wet, while little if any of the copper in Bordeaux was so absorbed. Apparently the copper absorbed by the leaves had little if any function in protection

TABLE 3.—*Effect of incubating sprayed plants in a moist chamber on the surface deposit of copper, and on the protective value of sprays for bean rust. (Plants sprayed with 0.1 per cent bluestone, with or without lime as indicated, in all cases)*

Copper determined or plants inoculated	Copper per square decimeter of leaves[a]		Reduction in number of rust pustules on inoculated plants[b]	
	Sprayed with bluestone	Sprayed with Bordeaux	Sprayed with bluestone	Sprayed with Bordeaux
	mg.	*mg.*	*Per cent*	*Per cent*
Immediately after spraying	0.56	0.50	79	100
After 4 hours in a moist chamber	0.24	0.50	56	99

[a] Average of 3 tests for each value.
[b] Average of 4 tests for each value.

from bean rust. Control was only slightly reduced by holding Bordeaux-sprayed plants in a moist chamber. It is apparent also that by holding bluestone-sprayed plants in a moist chamber disease control was reduced out of proportion to the loss of chemically determined surface copper. This is possible, because in addition to the absorption of surface copper by the leaves, nitrogenous materials, such as asparagin, may have diffused into the surface spray drops and reduced the protective value of the surface copper. In other tests, not reported here, the writer has found that the addition of asparagin to bluestone or Bordeaux decreased the protective value of these sprays for bean rust.

The above results further emphasize that an important function of lime in Bordeaux is to hold the copper in a form available and toxic to the rust fungus and relatively unavailable to the host.

VARYING THE COPPER : LIME RATIO

The studies reported so far have been concerned with the toxicant applied as bluestone, as lime, or as Bordeaux made from equal amounts of

bluestone and lime. Since it has been shown that Bordeaux has considerably more protective value per unit of copper than bluestone, it might be expected that there would be a gradual increase in protective value of bluestone with increasing amounts of lime. Average results of 2 tests to determine the effect of the copper : lime ratio on the protective value of Bordeaux for bean rust are presented in figure 3. With dosages of 0.001 and 0.003 per cent bluestone, disease control was slight, results were rather erratic, and no effect of lime at dosages from 0.001 per cent to 0.1 per cent was apparent. With 0.01 per cent, 0.03 per cent, and 0.1 per cent bluestone, however, control was marked with bluestone alone, and the amount of control increased as the lime was increased from none to 0.1 per cent lime. These tests were not sufficiently refined to determine accurately the optimum copper : lime ratio for best disease control under these conditions but indicate that it was of the order of about 1 : 1.

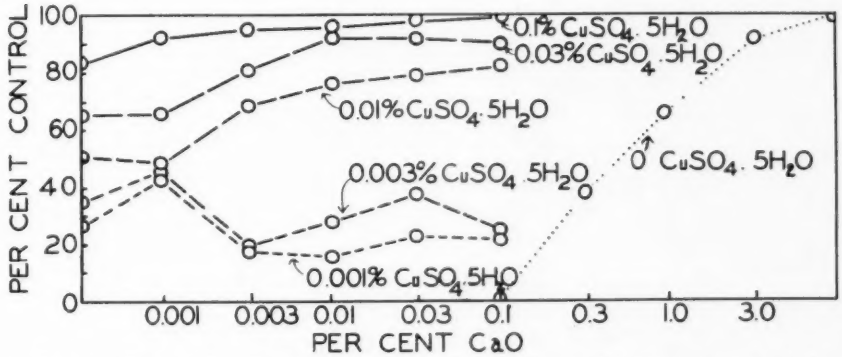

FIG. 3. Effect of varying the bluestone : lime ratio on the per cent control (= per cent reduction in number of rust pustules) of bean rust.

The effect of varying the bluestone : lime ratio on the eradicant value of Bordeaux was determined for bean powdery mildew. Average results of 2 tests, presented in figure 4, show that lime alone had very little eradicant action until a dosage of 0.1 per cent was reached; beyond this, powdery-mildew control was proportional to the dosage. Bluestone alone at 0.003 per cent caused about 38 per cent eradication; at 0.01 per cent, about 86 per cent; at 0.03 per cent, about 96 per cent; at 0.1 per cent, about 98 per cent; and at 0.3 per cent, 100 per cent. With all dosages of bluestone from 0.01 to 0.3 per cent the addition of increasing amounts of lime up to 0.3 or 1 per cent lime, caused a progressive reduction in the eradicant value of the spray. After 0.3 or 1.0 per cent lime was reached, an increase in lime caused an increase in control, but this increase apparently was due to the lime rather than the copper. Preliminary results, not presented here, show that bluestone applied as an eradicant spray for bean rust, a few hours after stomatal penetration had been effected, was more effective per unit copper than was Bordeaux. The depressing effect of lime on the eradicant value of blue-

stone for bean powdery mildew and bean rust is, therefore, apparently the opposite of its effect on the protective value of bluestone for bean rust.

DISCUSSION

From published observations, snapdragon rust (*Puccinia antirrhini*) is apparently similar to bean rust with respect to the role of lime and host leaves in the action of Bordeaux mixture as a protective fungicide. According to Doran (3) bluestone was about 100 times (magnitude not clear from Doran's data) as toxic per unit of copper to the urediospores on glass as was Bordeaux mixture. Doran used Bordeaux mixture without spreaders and found it was ineffective in controlling snapdragon rust, but Green (5) and Foster (4) added spreaders to Bordeaux and secured fair to excellent control of the disease.

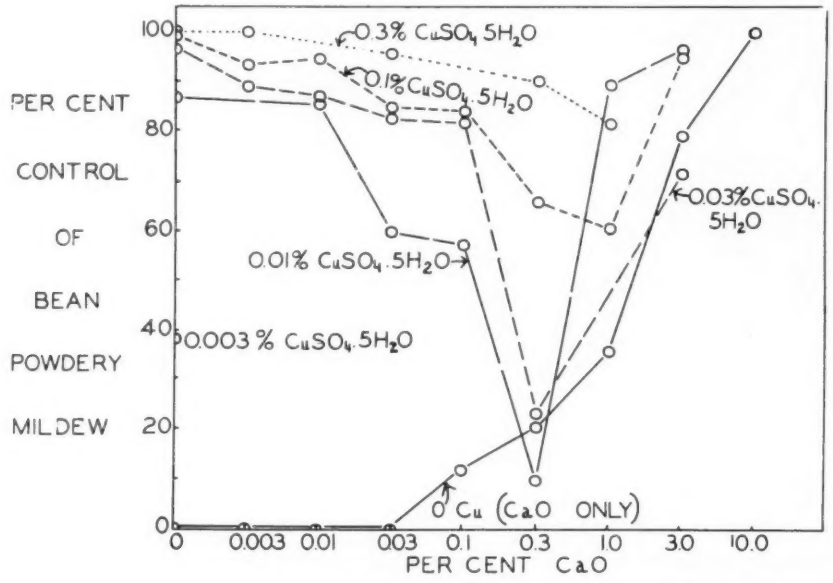

FIG. 4. Effect of varying the bluestone: lime ratio on the eradicant action of Bordeaux for bean powdery mildew.

With some fungi (Clark (2), and cucumber downy mildew in this paper) it would appear that Bordeaux mixture, on glass, and bluestone at the same copper dosage are about equally toxic. In the effect of the host in causing Bordeaux to be more active on leaves than on glass, as observed for bean rust, the writer knows of no comparable data in the literature.

Results reported here may have an important bearing on the application of *in vitro* toxicity to the evaluation of fungicides for the control of plant diseases. If many fungi behave as does bean rust, in that the same fungicide may be several hundred times more fungicidal on host surfaces than on glass, this would constitute a serious weakness to the *in vitro* testing of fungicides.

SUMMARY

Sprays consisting of lime alone, bluestone alone, and mixture of lime and bluestone (= Bordeaux mixture) were compared for their effect in inhibiting the germination of bean-rust urediospores and cucumber downy mildew sporangia, for protection from infection with bean rust and cucumber downy mildew, and for eradicating established infections of bean powdery mildew, at a range of dosages from below 50 per cent control to above 95 per cent control.

To give 95 per cent inhibition of germination of bean rust urediospores on glass required 300 mg. per sq. dm. of CaO, 0.60 mg. Cu as bluestone, or 64 mg. Cu as Bordeaux. To give 95 per cent reduction in number of rust pustules on plants required 124 mg. CaO, 2.6 mg. Cu as bluestone, or 0.18 mg. Cu as Bordeaux. For equivalent effectiveness it, therefore, required about 100 times as much copper in the form of Bordeaux as in the form of bluestone when the tests were on glass slides, but 10 times as much bluestone as Bordeaux when the tests were on leaves. To obtain equivalent effectiveness with Bordeaux, 350 times as much spray was required when the tests were on slides as when on leaves.

In similar tests with cucumber downy mildew, much less copper was required to inhibit germination than for bean rust, about the same amount of copper as bluestone or Bordeaux was required to give equivalent inhibition of germination on glass, and about the same amount of copper as bluestone or Bordeaux to give equivalent protection on plants, but much more copper was required to give 95 per cent protection than to give 95 per cent inhibition of germination. To give 95 per cent protection on leaves the addition of 0.05 per cent phthalic glyceryl alkyd resin as a spreader to the spray decreased the necessary amount of conventional fungicide to 27 per cent in case of lime, to 20 per cent in the case of bluestone, and to 2 per cent in case of Bordeaux.

Bean leaves sprayed with 0.1 per cent bluestone and held in a moist chamber for 4 hours showed by chemical determination only 43 per cent of the applied copper remaining on the surface of the leaves, and the control of bean rust was greatly reduced by this moist-chamber treatment, while there was no marked loss of copper or of rust control by holding Bordeaux-sprayed plants in a moist chamber under similar conditions. The results confirm other observations that leaves may absorb large quantities of copper from bluestone but not from Bordeaux.

When increasing amounts of lime were added to bluestone solutions the protective value of these mixtures for bean rust was progressively increased but the eradicant value for bean powdery mildew and bean rust was progressively decreased.

It is concluded that an important function of lime in Bordeaux mixture is to hold the copper in a form relatively unavailable and non-toxic to the host, but toxic to parasitic fungi, and an important function of the host may be to increase the effectiveness of the copper in some cases.

The relation of these results to the evaluation of fungicides is briefly discussed.

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TYPHULA SNOWMOLD OF PASTURE GRASSES[1]

C. C. WERNHAM[2] AND ST. JOHN P. CHILTON[3]

(Accepted for publication June 1, 1943)

In the spring of 1939 specimens of winter-killed Orchard grass, *Dactylis glomerata* L., collected at Storrs, Connecticut, by the junior writer, were examined. Fungus sclerotia embedded in the leaves and leaf sheaths indicated that low temperature alone may not have been responsible for all the damage. Low temperature, isolation plantings were made with these sclerotia. Two sclerotia-producing basidiomycetes were isolated, one very similar to *Typhula itoana* Imai, the other distinctly different. Cultures of these and other sclerotial fungi from turf grasses (5) were sent to Miss Remsberg (2) for identification. In her letter of April 4, 1940, Miss Remsberg wrote: "I am still working with the *Typhula* cultures you sent me. . . . It looks now as though #302 and #304 are *T. itoana,* #327 is a new species, . . . #303 looks familiar, but I'm not sure just which one it is yet." In addition to these cultures, authentic isolates of *Typhula* species were received from Miss Remsberg for comparative studies.

LIST OF CULTURES

Culture number	*Identification and record*
302	*Typhula itoana*—Akaroa Orchard grass. Storrs, Conn. Col. Chilton Apr. 1939; Isol. Wernham; Det. Remsberg.
303	*Typhula* sp.—Danish Orchard grass. Storrs, Conn. Col. Chilton Apr. 1939; Isol. Wernham; Det. Remsberg.
304	*T. itoana*—Danish Orchard grass. Storrs, Conn. Col. Chilton Apr. 1939; Isol. Wernham; Det. Remsberg.
308	*T. itoana*—Barley and wheat. Königsberg, Germany. Col. A. Volk; Det. Remsberg; Cornell Herb. 28090; Remsberg 93.
309	*T. itoana*—Barley and wheat. Sandpoint, Idaho. Col. Remsberg; Det. Remsberg; C. H. 27099; Remsberg 289.
310	*T. itoana*—Barley. Iwate Prefecture. Japan. Col. H. Tasugi; Det. Remsberg; C. H. 27095; Remsberg 283.
311	*T. itoana—Agrostis* sp. Ithaca, N. Y. Col. Chilton Apr. 1939; Isol. Wernham; Det. Wernham.
316	*T. itoana—Agrostis tenuis* Sibth. State College, Pa. Col. Isol. and Det. Wernham.

[1] Authorized for publication on March 1, 1943, as paper No. 1168 in the Journal Series of the Pennsylvania Agricultural Experiment Station and as contribution No. 53 of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, State College, Pennsylvania, in cooperation with the Northeastern States.

[2] Assistant Professor of Botany, The Pennsylvania State College.

[3] Formerly Agent of U. S. Regional Pasture Research Laboratory, now with the Department of Botany, Bacteriology and Plant Pathology, Louisiana State University, Baton Rouge, Louisiana.

324 *T. idahoensis*—*Agropyron cristatum* (L) Gaertn. Tetoma, Idaho. Col. C. W. Hungerford; Det. Remsberg; C. H. 27223; Remsberg 27.

325 *T. idahoensis*—Wheat. Bozeman, Mont. Col. P. A. Young; Det. Remsberg; C. H. 25153; Remsberg 291.

327 *Typhula* sp. *Agrostis* sp.—Wayzata, Minn. Col. L. J. Feser; Isol. Wernham; Det. Remsberg.

Unfortunately, Miss Remsberg never completed her identification of 303 and 327. At least they are not listed in her taxonomic treatment (2), nor in her pathological resume (3).

An investigation of *Typhula* snowmold of pasture and fine turf grasses was undertaken to ascertain: (a) A standardized method of inoculation under controlled conditions, (b) the host range of various isolates of *Typhula,* (c) the prevalence of physiogenic races, and, (d) the presence or absence of disease-resistant plants.

A preliminary experiment was devised to develop an inoculum and to determine the length of time necessary to obtain readily readable results from artificial inoculation.

EXPERIMENTAL RESULTS

Inoculum was prepared as follows: to 500 g. of wheat or rye, in wide-mouth two-liter flasks, was added 4 g. $CaCO_3$ and 500 cc. water. The flasks were plugged with cotton and autoclaved at 17 lb. pressure for 30 to 60 min. After cooling, each flask of medium was inoculated with sclerotia from potato-dextrose agar cultures kept at 13° C. The time of incubation at this temperature varied from 6 to 8 weeks, depending on the isolate.

Meanwhile seed of barley, oats, rye, orchard grass, timothy, Kentucky bluegrass, and Astoria bent was planted in pots of sterilized soil in a greenhouse. When the plants were well past the seedling stage they were inoculated.

Each flask of inoculum was emptied on clean paper and thoroughly mixed, then roughly divided into aliquot parts. A part of the sclerotia-infested grain was scattered uniformly over the plants in each pot and gently rubbed into the soil. Sterile sand was scattered over the plants and washed down until the inoculum was completely buried. Uninoculated, sand-covered checks were used with each culture. Pots were placed in a low-temperature chamber, maintained at 2 to 8° C., and watered regularly to simulate the moisture conditions under melting snow. Once a month they were examined for evidence of sclerotia in the culms and leaves. At the end of 4 months, when sclerotia began to appear, the plants were taken up and the amount of infection determined. Evidence of infection was considered to be "presence of sclerotia imbedded in culms and leaves" (Table 1).

There was considerable variation among strains and species of *Typhula* tested. This variation may have been due to (a) strain or species differences, (b) time elapse of incubation period, (c) stage of maturity of plants,

TABLE 1.—*Results from preliminary inoculation tests with Typhula sp. based on presence of sclerotia embedded in culms and leaves. Plants inoculated July 19. Data taken December 10, 1939*

Isolate	Number of seedlings diseased (D) or healthy (H) of																Total	
	Barley		Oats		Rye		Wheat		Orchard grass		Timothy		Kentucky blue		Astoria bent			
	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H	D	H
Typhula itoana																		
302	0	10	0	62	0	31	0	34	0	40	0	31	0	64	0	37	0	309
304	0	59	0	67	0	36	0	36	0	68	0	63	0	38	0	70	0	437
308	7	6	20	37	10	30	12	44	5	38	0	60	8	50	14	30	76	295
309	22	32	16	29	24	24	36	17	16	41	24	51	11	35	1	53	150	282
310	36	19	53	13	39	2	26	35	35	33	28	18	24	15	21	14	262	149
311	.....	.....	6	15	11	27	41	24	6	46	14	37	12	38	34	62	124	249
316	10	35	13	47	4	37	5	30	1?	54	14	32	0	31	13	59	59	325
Typhula idahoensis																		
324	0	45	0	40	0	26	0	66	0	43	0	46	0	30	0	48	0	344
325	0	52	0	63	0	48	2	52	0	35	0	45	0	37	0	85	2	417
Total	75	258	108	373	88	261	122	338	62	398	80	383	55	338	83	458	.....	.....
Checks	0	.....	0	.....	0	.....	0	.....	0	.....	0	.....	0	.....	0	.....	.....	.....

(d) variety of plant used, (e) the method of reading data, or (f) to experimental error. If the complete stock of isolates were to be tested more variation could be expected.

The method of inoculation and environment for incubation were least subject to change. Since, from our limited surveys, the injury occurring in pastures appeared to be largely due to *Typhula itoana, T. idahoensis* could be excluded in subsequent trials.

In the first of these trials 10 young plants from seed of each host species or variety were grown in rows in flats. Triplicate plantings were made for each culture of *Typhula* to be tested. The small-seed species were first germinated in sand and later transplanted to the flats. Randomized plantings were introduced to avoid position effect in the flat. Inoculations were made as indicated above and the flats were distributed at random in the cold chamber. Subsequent care was limited to watering the flats at the intervals necessary to keep the sand moist.

At the conclusion of the low-temperature exposure period the flats were removed to the greenhouse and allowed to dry out sufficiently to permit easy removal of sand and excess inoculum by jarring and shaking the flats. Each row of plants was removed and the individual plants were separated. When it was expedient to do so, the excess sand and soil particles were washed off by gentle manipulation in a bucket of water.

It became evident that in some cases particles of clinging soil and débris could be easily mistaken for sclerotia, even under a hand lens. In such doubtful cases of infection, resort was made to water immersion. The plant under consideration was immersed in water contained in the top or bottom of a glass moist chamber under which a sheet of white paper was placed. The ease of this method in distinguishing sclerotia from clay and small pebbles is worthy of mention. Most of the cultures could be disposed of at the rate of one flat of 110 plants, an hour; but a few cultures regularly consumed 4–6 hours per flat (Table 2).

The criterion "presence of imbedded sclerotia in culms and leaves is evidence of infection" failed to give the true picture under the conditions of this experiment. Flats inoculated with cultures 302 and 304 were tedious to read. Sclerotia in the tissues were hard to find; when they were located it was often apparent that they occurred in portions of leaves buried under the sand. Mere saprophytism could be responsible for their presence. The abundance of sclerotia produced by all other isolates on all parts of the plants, even on the roots of the cereals (1, 4), seemed to indicate a positive parasitism. Remsberg (2), p. 71, cites *Dactylis glomerata* as a host of *Typhula itoana*. Except for cultures 302 and 304, our data confirm her findings. It is possible that these two strains were saprophytic. If this were true, then culture 303 may have been the true orchard-grass pathogen at Storrs, Connecticut, or the winter killing was purely physiogenic. In this experiment the cereals were invariably killed and often disintegrated to the point of being irrecoverable. The grasses in most cases were injured externally, but the crowns appeared to be viable.

TABLE 2.—*Results of inoculation tests of Typhula species on various grains and grasses, from three randomized plantings of 10 plants each, expressed as number of infected plants of total plants recovered. Inoculated May 23, 1940. Data taken October 8, 1940*

Hosts	Planting date	Number of seedlings infected (I) and total (T) with indicated isolate number														Total	
		302[a]		304[a]		308		309		310		316[b]		327[b]			
		I	T	I	T	I	T	I	T	I	T	I	T	I	T	I	T
Bent, Astoria	2–28–40	29	29	22	30	27	27	27	30	26	26	30	30	29	29	190	201
Bent, Colonial	2–28–40	27	30	28	30	30	30	28	29	28	28	29	29	21	28	191	204
Barley	4–26–40	14	27	11	28	23	28	23	28	29	29	22	26	12	30	134	196
Kentucky bluegrass	3– 8–40	24	29	23	27	29	29	27	27	30	30	22	25	14	25	169	192
Oats	4–26–40	20	26	13	29	29	29	27	29	27	28	24	27	17	29	157	197
Orchard grass	2–28–40	24	30	22	28	29	29	29	29	29	29	30	30	21	28	184	203
Orchard grass, Danish 22385	3–11–40	22	25	16	26	19	19	24	25	26	26	28	29	26	29	161	179
Orchard grass, Akaroa 118759	2–28–40	23	26	25	30	30	30	26	26	26	26	29	29	16	26	175	193
Rye	4–26–40	16	22	13	25	30	30	21	25	27	27	18	19	11	21	136	169
Timothy	2–28–40	23	27	16	27	25	29	23	29	27	27	27	27	14	29	155	195
Wheat	4–26–40	21	30	16	28	28	29	24	30	30	30	23	27	10	30	152	204
Total	.....	243	301	205	308	299	309	279	307	305	332	282	298	191	304	.....	.....

[a] Sclerotia very scarce and hard to find; if present chiefy on buried leaves.
[b] Specimens of each infected host deposited in botany herbarium.

On the basis of the above results a third experiment was planned. The following features were incorporated: (1) Inclusion of a noninoculated check for the estimation of plant viability as affected by the treatment, (2) trimming-off of all excess leaf material previous to inoculation, (3) application of sand in such a manner that a minimum of plant tissue was buried, (4) more care taken to insure a perfect stand, and (5) incorporation of culture 303 into the test.

Unfortunately, such a careful set-up ended in near disaster, due to the fact that peat was used to conserve moisture in the flats and damping-off resulted. Much transplanting had to be done and a very uneven stand was obtained. In addition the inoculum became contaminated from standing too long. Because of the time consumed in preparation, it was decided, however, to salvage what remained and make the tests (Table 3). Certain

TABLE 3.—*Results of inoculation tests of Typhula species on various grains and grasses, from three randomized plantings expressed as number of infected plants of total plants recovered. Inoculated April 1, 1941. Data taken August 14, 1941*

Host	Number of seedlings infected (I) and total (T) with indicated isolate No.						Total		Check	
	303		310		327					
	I	T	I	T	I	T	I	T	I	T
Bent, Astoria	30	30	24	29	31	31	85	90	0	30
Bent, Colonial	29	29	23	29	30	30	82	88	0	30
Barley	33	34	33	34	32	32	98	100	0	31
Ky. bluegrass	30	30	28	30	28	30	86	90	0	29
Oats	27	29	33	34	29	30	89	93	0	30
Orchard grass	32	32	29	30	30	30	91	92	0	30
Orchard grass, Danish 22385	30	30	30	30	30	30	90	90	0	30
Orchard grass, Akaroa 118759	29	29	28	30	30	30	87	89	0	30
Rye	16	22	20	24	28	30	64	76	0	26
Timothy	30	30	26	30	30	30	86	90	0	30
Wheat	30	30	29	31	28	29	87	90	0	30
Total	316	325	303	331	326	332	.....	.....	0	326

positive results were obtained. The treatment had little effect on the viability of the uninoculated check plants. The clipped and trimmed plants were abundantly covered with sclerotia. Culture 303 was equal in pathogenicity to 310 and 327.

The meager results obtained from this experiment left the peculiar results of the other two orchard grass isolates, 302 and 304, unexplained. This reaction was a general one applicable to all plants tested rather than a specific one limited to Orchard grass alone. Nor did it resemble the reactions of other cultures of *Typhula* on plants from the same species. Unfavorable temperature or time of exposure could have been responsible for the results. Of these factors, the latter offered the most promise for further tests. Varying the temperature involved too much space and equipment for the length of time required. For this reason it was decided to limit a final experiment

TABLE 4.—*Results of inoculation tests of Typhula species on various grasses, from two random plantings of 10 plants each expressed as number of infected plants of total plants recovered. Inoculated February 23, 1942. Data taken September 23, 1942*

Hosts	Number of seedlings infected (I) and total (T) with indicated isolate numbers																Total		Check	
	302[a]		303[b]		304[a]		308		309		310		316		327					
	I	T	I	T	I	T	I	T	I	T	I	T	I	T	I	T	I	T	I	T
Kentucky bluegrass	17	20	18	18	15	20	20	20	19	20	20	20	20	20	20	20	149	158	0	19
Orchard grass	18	20	20	20	18	20	19	19	18	19	20	20	20	20	19	19	152	156	0	20
Orchard grass, Danish 22385	19	19	19	19	20	20	19	19	20	20	20	20	20	20	20	20	157	157	0	20
Orchard grass, Akaroa 118759	19	19	20	20	19	19	17	17	20	20	20	20	18	18	19	19	152	152	0	19
Timothy	12	20	18	18	12	14	20	20	20	20	20	20	20	20	19	19	141	151	0	20
Total	85	98	95	95	84	93	95	95	97	99	100	100	98	98	97	97	.....	.....	0	98

[a] Sclerotia small and relatively sparse, though more numerous than in previous trials.
[b] Specimens on all hosts deposited in botany herbarium—very pathogenic.

to the time variable, to include only grasses and to limit these to duplicate plantings (Table 4).

In spite of the lengthened exposure (7 months instead of 4.5 months), the check plants remained remarkably viable. Inoculated plants were nearly all killed. Cultures 303, 308, 309, 310 and 316 caused greatest injury. This was easily determined without disturbing many plants. Least destructive were cultures 302, 304 and 327. Of these 302 and 304 showed a slight increment in number of sclerotia present, but these were small compared to their size in culture.

DISCUSSION

The particular value of the work herein reported lies in the fact that most pasture grasses have proved quite susceptible to the species of *Typhula* commonly found on grains and grasses (2). Under the conditions of our experiment there was very little evidence of disease resistance, although some noninfected plants usually were found. The small number of plants used in these tests hardly serve as a basis for determining this character. That disease resistance occurs in some species has been reported by Remsberg and Hungerford (4) and demonstrated by Wernham (5). The importance of disease resistance is evident in any grass-improvement program provided that the disease is economically serious in the area. The extent of winter injury in pastures is not very well known, probably because the pastures are not examined early enough in the season. It is impossible, therefore, to estimate the percentage of winter injury that is due to disease. It seems logical from the data presented that the amount and severity of winter injury due to snowmold would depend on the type and duration of certain weather phenomena. A dry, open winter would present less favorable conditions than a wet, snowy one of long duration. The senior writer has observed this to be the case in field-plot inoculations on fine turf.

Whatever importance snowmold of pasture grasses may assume, an experimental approach to the study of disease resistance has been established under conditions of artificial inoculation. Independent of the vagaries of the weather to which field-plot techniques are subjected, the method provides for positive results at any season on a scale of operation hitherto unattained (4). In addition it suggests that artificial covering may be substituted for snow in field-plot trials.

The evidence indicates, too, that not all the organisms responsible for winter killing or injury have been determined. Nor is it safe to assume that the presence of a familiar looking organism is evidence of its pathogenicity, regardless of its previously established record. There does not seem to be any evidence of pathogenetic specialization among the species of *Typhula* tested.

SUMMARY

A method of testing grasses and cereals for their reaction to *Typhula* sp. under controlled conditions is described.

An undescribed species of *Typhula* is probably responsible for some winter killing of Akaroa and Danish orchard grass.

The more common pasture grasses of the Northeastern States are highly susceptible to *Typhula* snowmold under artificial conditions.

Most of the isolates of *Typhula* sp. tested herein are pathogenic to all the species of grasses included in the tests.

Pathogenetic races among the species of *Typhula* considered were not revealed under the conditions of our experiments.

A statement regarding the presence or absence of plants resistant to *Typhula* sp. is not warranted from our results.

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SOIL FUMIGATION WITH CHLOROPICRIN FOR CONTROL OF THE ROOT KNOT NEMATODE, HETERODERA MARIONI[1]

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INTRODUCTION

It is a well-recognized fact that the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, is one of the worst plant pests known. The possibility of control of this pest by means of volatile poisons, applied to the soil before planting a host crop susceptible to damage, has long been recognized, and numerous chemicals have been more or less thoroughly tested for this purpose. One of the most promising of these is chloropicrin.[2]

REVIEW OF LITERATURE

Chloropicrin was first employed as a nematocide in 1919 (11), but little was published on the subject until 1932. In that year Johnson and Godfrey (10) reported that good control of root knot could be obtained by the use of 163 lb. of the chemical per acre. In their experiments it was applied by introducing small amounts into holes in the soil, 5 or 6 inches deep and 18 inches apart. In 1934, Godfrey, Oliveira, and Hoshino (8) reported that good control of root knot had been obtained in Hawaii by applying 250 to 400 pounds of chloropicrin per acre to loose soil without excessive moisture. Applications were spaced no more than 18 inches apart, and a gas-impervious cover of glue-coated paper was placed over the soil after treating. In 1935, Godfrey (6) reported that applications of 130 to 170 lb. of chloropicrin per acre in pineapple fields had given sufficiently good control of root knot to produce a substantial increase in yield. Neller and Allison (12) in 1935, designed a machine for the rapid application of chloropicrin and found that good control of root knot was obtained by the use of 203 and 405 pounds of chloropicrin per acre. Newton, Bosher, and Hastings (14) devised a method for determining spacing of injections of soil fumigants in 1937 and obtained fair control of root knot in greenhouse benches by the use of 1 ml. of chloropicrin per square foot.

In 1939, Howard, Stark, and Smith (9) reported that surface watering of the soil was as effective as gas-impervious covers in increasing the efficacy

[1] Cooperative investigations by the Division of Nematology, Bureau of Plant Industry, United States Department of Agriculture, and the Georgia Coastal Plain Experiment Station, Tifton, Georgia.

[2] Chloropicrin (CCl_3NO_2) is a colorless liquid with a specific gravity of 1.63. It vaporizes easily, and the vapor is extremely irritating to the eyes, causing them to water profusely. Larger concentrations taken into the lungs cause violent coughing, nausea, and vomiting. These effects are produced by concentrations far below the lethal point and are only temporary, usually being relieved after a few minutes in fresh air. The liquid is highly irritating to the skin, and care should be taken to protect the hands and face while handling it. If spilled on clothing, the garment affected should be removed at once. It is not inflammable or explosive. If the user transfers it from one vessel to another only in the open air and takes ordinary precautions, it is not particularly dangerous to handle. However, any person susceptible to or afflicted with a respiratory disease should use full-face gas-mask protection, even while working out of doors.

of chloropicrin fumigations. In the same year, Chitwood (1) further refined the method of determining soil spacing for fumigants, and Taylor (16) developed mathematical formulas for calculating optimum amounts for use. In 1940, Young (18) reported good control of root knot by the use of 300 to 600 lb. of chloropicrin per acre, and found that several sorts of glue were suitable for coating paper to use in covering the soil after treatment. In the same year Chitwood (2) reported that root knot had been controlled in greenhouses and in the field by placing doses of a mixture containing 1.5 ml. of chloropicrin and 8.5 ml. of ethylene dichloride[3] in the soil in holes 10.5 inches apart in rows 9 inches apart. Taylor and McBeth (17) reported in 1941 that root knot of watermelons could be greatly reduced in the field by means of "spot treatments," involving only the small portion of the soil occupied by the roots of the plants, with considerable economy of material. Also in 1941, Chitwood (3) reported that root knot and fungus diseases of gerberas in greenhouse soil, and root knot and fungus diseases of tomatoes in the field, could be controlled by the mixture of chloropicrin and ethylene dichloride mentioned above.

Chitwood and Newhall (4) reported in 1942 that fall applications of chloropicrin, at the rate of 2 ml. per hole, with the holes spaced 10.5 inches apart in the soil, or a mixture of 1 part chloropicrin and 9 parts ethylene dichloride applied at the rate of 10 ml. to the hole, with the same spacing, gave good control of onion bloat caused by the nematode *Ditylenchus dipsaci* (Kühn) Filipjev. In this year also, Newhall and Stark (13) reported excellent control of *Heterodera marioni* on a spring crop of tomatoes by treating the soil the previous fall with chloropicrin at the rate of 10.5 pounds per 1000 square feet (460 pounds per acre) or with a 1 : 9 mixture of chloropicrin and ethylene dichloride at the rate of 1,824 pounds per acre. Both were applied at 10-inch intervals in holes 4 to 5 inches deep, 2-ml. doses of chloropicrin or 10-ml. doses of the mixture being used. Chloropicrin has also been found to be highly effective against insects (15) and fungi (7) in the soil.

EXPERIMENTS

The experiments reported herein were begun in the summer of 1936 for the purpose of testing results obtained by previous investigators and developing a dependable method of using chloropicrin against the root-knot nematode. No other plant-parasitic nematodes, insects, fungi, or bacteria were considered.

In all the work reported, the soil used was Norfolk sandy loam in a field near Tifton, Georgia, that had been used several years for root-knot-susceptible crops and had a moderate infestation of root-knot nematodes. All experiments were performed under outdoor conditions during the spring, summer, and fall.

[3] Ethylene dichloride ($C_2H_4Cl_2$) vapors are highly toxic, inflammable, and explosive. Persons handling this substance should be extremely careful of fires and should wear full-face gas-mask protection.

Basic procedure was the same in all cases. The soil was prepared by plowing and leveling, the chloropicrin injected about 6 inches beneath the soil surface by means of carefully calibrated applicators, especially designed for the purpose, and the remaining soil population determined by use of the indicator-plant method. This method was tested by Godfrey (5), who recommended that a quick-growing crop, highly susceptible to root knot be planted at the rate of one plant per square foot on the soil to be tested. The plants are allowed to grow long enough to be invaded by most of the nematodes in the soil but not long enough to allow reproduction and secondary infection to occur. This period is about 30 days when soil temperatures are above 20° C. The roots are then removed with ordinary care, examined, and classified as infected or not infected. The correlation between the percentage of infected plants and the nematode population of the soil is sufficiently high to enable fairly accurate estimates of the soil population to be made and certainly high enough to show the large changes in population that might be expected to follow successful control measures. The system used in the present study followed this method, except that indicator plants often were allowed to grow from 6 weeks to 2 months, permitting the development of secondary infection and increasing the chances of detecting very small populations.

Precautions were taken during the growing period to prevent contamination of the plots by nematodes brought in by running water and cultivating tools. Border effects due to migration of nematodes into the plots were eliminated by examining only plants from the central portions of the plots and rejecting those growing within 2 feet of the edge. Indicator plants were invariably examined with a dissecting microscope and were classified as infected if even one root-knot nematode was found. Fifty to 100 plants were examined from each plot.

Results of the experiments are given in the tables as percentages of infected indicator plants. Differences in averages are referred to as "significant" when statistical analysis of the results indicated that odds were at least 19 to 1 that results were not due to chance, and as "highly significant" when the odds were 99 to 1 that results were not due to chance.

The chloropicrin applied was the standard commercial grade (99 per cent pure). Applications always were spaced so that adjacent application points formed equilateral triangles, and the application distances given are the length of the sides of these triangles (16). Soil temperatures were always measured at a depth of one foot. Diurnal variation in soil temperature at this depth usually is only 1° to 2° C. in southern Georgia.

Experiment No. 1 consisted of 25 plots, each 16 by 16 feet, arranged in a Latin square. Rates of application of the chloropicrin were: None, 50, 100, 200, and 400 lb. per A. Application points were spaced 16 inches apart. The soil temperature at the time of treatment was 25° C. Soil moisture was not determined. Plots were covered with overlapping strips of mulch paper immediately after treating and uncovered 4 days later. Ex-

amination of the Whippoorwill cowpeas, *Vigna sinensis* (L.) Haussk., used as indicator plants, gave the results presented in table 1.

TABLE 1.—*Percentages of root-knot infected indicator plants in plots treated with chloropicrin and covered with mulch paper*

Treatment (pounds chloropicrin applied per acre)	Infected plants in plots after application of chloropicrin					
	Row 1	Row 2	Row 3	Row 4	Row 5	Average[a]
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
0	56	50	27	14	5	30.4
50	17	7	1	2	0	5.4
100	5	5	0	0	0	2.0
200	0	0	0	0	0	0.0
400	0	0	0	0	0	0.0

[a] Differences between control and all treatments significant; no significant difference between treatments.

Experiment No. 2 was a 25-plot Latin square treated with chloropicrin at the following rates: None, 50, 100, 150, and 200 lb. per A. Application points were spaced 16 in. apart. The soil temperature at time of treating was 20° C. Soil moisture content was not determined. No paper covers were used after treatment. Instead, the plots were sprinkled with enough water to wet the top inch or two of soil, forming a "water seal" to prevent too rapid escape of the fumes. Squash (*Cucurbita maxima* Duchêsne) was used as an indicator crop. Results are given in table 2.

TABLE 2.—*Percentages of root-knot infected indicator plants in plots treated with chloropicrin and water-sealed*

Treatment (pounds chloropicrin applied per acre)	Infected plants in plots after application of chloropicrin					
	Row 1	Row 2	Row 3	Row 4	Row 5	Average[a]
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
0	86	32	30	36	68	50.4
50	45	18	30	12	8	22.6
100	29	41	2	11	8	18.2
150	3	4	0	1	3	2.2
200	2	6	0	4	2	2.8

[a] Differences between control and all treatment averages highly significant; difference between 100-lb. and 150-lb. treatments significant.

Experiment No. 3 consisted of 40 randomized plots, each 16 by 16 feet. Chloropicrin was applied to 32 of these in 4 groups of 8 plots each at the rates of 50, 100, 150, and 200 lb. per A.; and the remaining 8 plots were left untreated. As the experiment was originally designed to explore the relation of soil temperature and moisture to chloropicrin fumigations, groups of plots were treated at various temperatures from 18° to 25° C. and at soil moistures ranging from 2 to 8 per cent. Spacing of applications was 16 inches. No cover or water seal was used. As there were no significant

differences in the temperature and moisture portions of the experiment, table 3 gives the percentages of infected Whippoorwill cowpea indicator plants grouped according only to amounts of chloropicrin used.

As the only variation in technique between this experiment and the two previously described was the omission of the cover or water seal, the results indicated that successful fumigation cannot be expected when neither of these is used.

In another experiment a maximum of 200 lb. of chloropicrin was applied to soil having a moisture content of only 2 per cent. Although glue-coated kraft paper covers, with the joints lapped 6 inches and glued and the edges buried 8 inches in the soil, were used, no appreciable effect on the root-knot nematode population of the soil was produced.

TABLE 3.—*Percentages of root-knot infected indicator plants from plots treated with chloropicrin without cover or water seal*

Treatment (pounds chloropicrin applied per acre)	Infected plants in plots after application of chloropicrin								
	Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Block 7	Block 8	Average[a]
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
0	36	57	49	26	12	31	37	33	35.1
50	24	62	5	27	57	42	65	49	41.3
100	49	1	38	63	67	45	31	26	40.0
150	1	33	82	60	52	42	31	25	40.7
200	31	6	42	0	8	37	58	33	26.8

[a] No significant differences.

That chloropicrin might have important effects on germination of seed under certain conditions was demonstrated by an experiment in 1940. A block of plots was treated with chloropicrin at rates of 200, 300, and 400 lb. per A., with 4 plots to each treatment and 4 nontreated control plots. Soil moisture at time of treating was only 6 per cent, but some rain fell on each of the 8 days after the chemical was applied. An indicator crop of cowpeas, planted 5 days after treating, failed to emerge on the plots receiving 400 lb. of chloropicrin per acre. Approximately 10 per cent of the seed on the 300 lb. per A. plots germinated, 26 per cent on the 200 lb. per A. plots and 80 per cent on the nontreated checks. A second indicator crop, planted 1 month after treating, grew normally. Apparently normal escape of the fumes from the soil had been prevented by the daily rains, and enough remained when the first indicator crop was planted to injure the seed.

The experience gained in the previous work made possible the formulation of a system of using chloropicrin, which was tested in 1940. The procedure in detail was: The plots were plowed, harrowed, and leveled, so that the soil was well broken up and very loose at the time of treating. Soil temperature was 21° C. and soil moisture was 6.7 per cent. Sixteen 10 by 10 foot plots were laid out, raked smooth, and lightly sprinkled with water.

The soil surface was marked off in a grid of rectangles, 12 by 14 inches in size. The applicator was set to place the chemical 6 inches beneath the soil surface. Working along the lines 12 inches apart, the measured applications were placed at the intersections of the marked lines on the odd-numbered rows and halfway between these intersections on the even-numbered rows. Holes made by the applicator spike were covered with soil by the operator immediately after the chemical was placed. When chloropicrin had been applied to the whole plot, it was again raked and sprinkled with enough water to wet the top inch or two of soil. This completed the treatment. The plots were left undisturbed for a week to allow escape of the chemical, and then planted.

The 16 plots were randomized according to the Latin square method, and 4 plots assigned to each of the following treatments:

(A) No chloropicrin.

(B) 1.1 ml. of chloropicrin per application point (150 lb. per A.).

(C) 1.4 ml. of chloropicrin per application point (200 lb. per A.).

(D) 1.8 ml. of chloropicrin per application point (250 lb. per A.).

Squash were used as indicator plants and examined 6 weeks after planting. Results of the examination are presented in table 4.

TABLE 4.—*Percentages of root-knot infected indicator plants from plots treated with chloropicrin in 1940. Applications spaced at 14-inch intervals and water seal used*

Treatment (pounds chloropicrin applied per acre)	Infected plants in plots after application of chloropicrin				
	Row 1	Row 2	Row 3	Row 4	Average[a]
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*
0	95	93	79	74	85.2
150	10	7	17	19	13.2
200	3	0	0	0	0.7
250	0	2	0	0	0.5

[a] Differences between control and all treated plots highly significant; differences between 150- and 200-pound treatments significant.

In the experiments reported above, the chloropicrin was applied when the soil contained no undecayed roots, as it was presumed that these would protect the contained nematodes from the effects of the fumigation. In commercial greenhouses, the time required for the decay of the roots of one crop, before fumigating the soil and planting another is often very important. An experiment designed to test the effect of undecayed roots in the soil on the efficiency of chloropicrin fumigation was performed in 1942. An infested plot, 50 by 50 feet, was planted to tomatoes (*Lycopersicon esculentum* Mill.), which were allowed to grow for 6 weeks. At the end of this period, the main roots of the plants were heavily infected with root knot, with many large galls 1 or 2 cm. in diameter. The plot was then plowed, covering the tomato plants completely. On the same day, the plot was sub-

divided to form 25 plots, each 10 by 10 feet. These were randomized by the Latin square method, with 5 plots in each of 5 treatments. The first group of plots (A) was the nontreated control. Chloropicrin at the rates of 200 and 300 lb. per A. was applied, on the same day the plots were plowed, to the second group of plots (B) and the third group of plots (C), respectively. Twenty days later, when the tomato roots had entirely disintegrated, the fourth (D) and fifth (E) groups of plots were treated with 200 and 300 lb. of chloropicrin per A., respectively. Thus the experiment consisted of control plots, plots treated when the roots were fresh, and those treated when the roots had entirely decayed, with chloropicrin applied to each of the latter two groups at 200 and at 300 lb. per A. Treatment procedure was as described for the preceding experiment. An indicator crop of cantaloupes (*Cucumis melo* L.) was planted and examined after growing for 1 month. Results of the examination are given in table 5.

TABLE 5.—*Percentages of root-knot infected indicator plants (cantaloupes) from plots treated with chloropicrin and containing infected tomato roots*

Treatment (pounds chloropicrin applied per acre) and condition of tomato roots	Infected plants in plots after application of chloropicrin					
	Row 1	Row 2	Row 3	Row 4	Row 5	Average[a]
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
(A) None	86	96	100	96	98	95.2
(B) 200 (Roots fresh)	0	27	78	54	26	37.0
(C) 300 (Roots fresh)	0	20	28	2	8	11.6
(D) 200 (Roots decayed)	2	6	12	0	0	4.0
(E) 300 (Roots decayed)	0	0	6	0	0	1.2

[a] Difference between control plots (A) and all treated plots highly significant; difference between B and C significant; differences between C, D, and E not significant.

DISCUSSION AND CONCLUSIONS

From the practical standpoint, satisfactory control of the root-knot nematode might be defined as an effected reduction in the soil population of this nematode to the point where at least 90 per cent of the indicator plants escape infection in the early stages of growth. Under these circumstances, about a year would be required for the number of nematodes again to reach the point where reductions in crop yields would be noticeable, even if the soil were used continuously for highly susceptible crops.

The results of the experiments indicate that this degree of control can be obtained under Tifton, Georgia, conditions by the use of a minimum of 200 pounds of chloropicrin per acre. Insignificantly better results were obtained with larger amounts. Smaller applications did not always give satisfactory control.

The condition of the soil at the time of application and the use of some device to confine the fumes to the soil are as important as the amount of chloropicrin used. Fumigation is effective within the entire soil-temperature range tested, 20° to 27°; furthermore, data from experiments not reported here indicate that successful fumigations can be made at temperatures even as low as 15° C. Fumigations made when the soil was moderately damp (moisture content about 6 per cent) were good, but those made when the soil was very dry (moisture content 2 per cent) were not successful, even when covers were used. While no data are available, it is probable that excessive soil moisture would prevent successful soil fumigation, since the diffusion of the fumes through the soil interspaces would be hindered by the water.

Fumigations made without the use of either paper covers or water seals were not successful. Mulch paper, and wetting the top inch or two of soil with water were effective in confining the fumes.

Results of the experiment with undecayed roots indicate that these interfere seriously with the efficiency of the fumigation. Where 200 pounds of chloropicrin per acre were used, satisfactory control was not obtained when fresh roots were in the soil, although the reduction in population produced by this amount after the roots had disintegrated was very satisfactory. When the amount of chloropicrin was increased to 300 pounds, a fair degree of control was obtained, even with undecayed roots in the soil. However, control in this case would be considered a little less than satisfactory, since two of the plots had 20 per cent or more of infected indicator plants.

In the sandy loam soils used in these experiments, applications can be spaced at 14- to 16-inch intervals. It is safe to assume that chloropicrin would be effective wherever the soil is open enough to permit ready diffusion of the fumes, but it is probable that optimum spacing and amounts would vary greatly. For this reason, general recommendations cannot be made. Prospective users are advised to experiment on a small scale before attempting large and extensive applications.

In one experiment, seeds planted before all of the chloropicrin had diffused out of the soil were injured, but this case was exceptional. No injury was observed to seeds planted one week after treating in the 1940 experiment.

Fumigation of one acre of soil with 200 pounds of chloropicrin would cost $160 for the chemical alone at minimum present prices. Application requires about 1 man-hour per 1,000 square feet of soil when a hand applicator of efficient design is used. It is apparent that the use of chloropicrin is economically practical only where crops of high value are to be grown. In greenhouses and seedbeds, it can replace steam sterilization in some cases or can be used where steam is not available. Other uses might be found in vegetable gardens, in seed beds, or in ornamental flower beds. In the field, only crops of high unit value would repay the investment. Here considerable savings may be made by the use of the spot-treatment technique (17). In this system, only the portion of the soil to be occupied by the roots of the

plants is fumigated. Cost of the chemical for watermelons in hills spaced 8 feet apart is about $5 per acre. Cost for other crops would vary according to the proportion of the soil occupied by the plant roots. Apparently it is impractical to eliminate the nematodes entirely, so the treatment would have to be repeated periodically, possibly every year where the soil is used continuously for susceptible crops.

SUMMARY

The nematocidal effect of chloropicrin was tested in the field against the root-knot nematode, *Heterodera marioni* (Cornu) Goodey. Satisfactory control was obtained by the use of 200 pounds of the chemical per acre in sandy loam soil containing no undecayed roots. Applications were made by placing measured quantities of chloropicrin in holes in the soil 6 inches deep and spaced at 14- to 16-inch intervals. Immediately after treating, the soil was sprinkled with enough water to wet the top inch or two, or was covered with mulch paper or glue-coated kraft paper. Applications made when the soil-moisture content was only 2 per cent or when no cover or water seal was used failed to produce satisfactory control. Amounts of chloropicrin less than 200 pounds per acre did not always produce satisfactory results.

When the soil contained fresh infected tomato roots, poor control was obtained with 200 pounds of chloropicrin per acre and fair control with 300 pounds per acre. In the same experiment, 200 pounds per acre applied after the infected roots had disintegrated, produced good control.

Germination of seed was partly prevented in one experiment where the indicator crop was planted before the chloropicrin had fully escaped from the soil.

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SOME CULTURAL PRACTICES THAT INFLUENCE THE DEVELOPMENT OF ALTERNARIA SOLANI ON TOMATO SEEDLINGS[1]

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INTRODUCTION

A large part of the tomato canning-crop acreage of the United States is planted with seedlings that are produced in open field beds. This type of culture has introduced some important factors such as soils, temperature, humidity, fertilization, and methods of handling and transportation, which affect both the vigor of the plants and the problem of disease control. *Alternaria solani* causes one of the most important diseases affecting tomato seedlings in the area where they are grown in open fields. Recent investigations indicate that there are several conditioning factors involved in tomato seedling production under open field conditions that, if overlooked, can materially influence the amount and severity of infection by this fungus in any given season.

The production of tomato seedlings in the South for shipment to the North has developed into a large industry, particularly important to both growers and canners. Although playing an important role in the canning industry, field production of tomato seedlings has reached its present state without much research information on the specialized cultural practices involved, particularly as they may influence the development and growth of the plant after it is set in the field. As a result of field tests conducted in the plant production area of Georgia, Hester (2) showed the importance of certain soil and fertilizer practices in growing tomato seedlings. He also suggested the inclusion of plant nutrients in the packing moss in order to maintain plant vigor during shipping. Hartman and Stair (1) made comparative studies of plants from various sources, but were interested primarily in plant growth and subsequent yields. They concluded that plant condition at the time of setting was more important than the locality from which they came. In an effort to maintain plant vigor, Sayre (4, 5, 6) found the use of nutrient "starter" solutions at the time of transplanting beneficial both in promoting rapid early growth and in the production of larger yields. Seaton and Strong (7) compared southern field-grown plants with Michigan cold-frame plants and concluded that the southern plants

[1] Conducted as a phase of cooperative investigations between the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture; the Department of Botany, Indiana Agricultural Experiment Station; the New Jersey Agricultural Experiment Station; the Georgia Coastal Plain Experiment Station; the Georgia Agricultural Experiment Station; and the Georgia State Department of Entomology.

[2] Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

were "harder" and tended to develop more disease than their own locally grown plants. During the course of tomato seedling-disease investigations, conducted by the writers in Georgia and in Indiana from 1937 to 1942, it was found that 3 factors in the production and handling of tomato seedlings especially influence the development of *Alternaria solani* on commercially grown plants. These factors are: age of plants, wilting of plants, and length of time in transit or storage. A discussion of these, together with supporting data, is presented in this paper.

DISEASE INCIDENCE AS AFFECTED BY AGE OF PLANTS

Field-grown tomato seedlings are considered to be of the best shipping size when the height is from 6 to 9 inches. Under normal growing conditions the plants in a given field will stay within these limits for only a few days. The plants must be harvested during this critical period, or they become oversized, hard, and fibrous. Field observations have indicated that infection by *Alternaria solani* becomes appreciably greater when plantings reach this latter stage of growth, and critical buyers often refuse shipments of such plants. In order to determine the relation of plant age to *Alternaria solani* incidence, successive plantings of treated Marglobe tomato seed were made each year from 1939 to 1942. Dates of planting were as follows: 1939, March 8, 18, 28; 1940 and 1941, March 10, 20, 30; and 1942, March 11, 23, and April 2. Plots 25×50 feet were used for each planting, the rate of fertilizer application, number of rows, and rate of seeding being the same in all cases. In order that the disease might develop normally, no fungicidal sprays or dusts were used on the seedlings. As the plants in each planting reached commercial shipping size, 5 random samples of 25 plants each were pulled and packed according to commercial practice. Precautions were taken at each harvesting to pull only plants of acceptable commercial size and quality. After a storage period of 48 hours, the plants were transplanted to plots in another section of the field. This was repeated at 4- to 10-day intervals until 3 harvests had been made on each planting. After a growth period of 14 to 18 days, each transplanting was pulled, washed, and the total number of stem cankers per sample of 25 plants was recorded. Due to a lack of canker development in all plots in 1939, only leaf-spot data were taken, the readings being made in the field before pulling the seedlings. The experiments in which the plants were stored and then transplanted were conducted from 1940 to 1942.

The combined stem-canker and leaf-spot data for the 3 harvest dates during the period of these experiments are shown in table 1. Due to varying humidity conditions between both plantings and harvests, the data are not consistent in all cases. The plants did, however, tend to increase in susceptibility to infection as they became older. Of particular importance is the fact that this relationship persists regardless of the date of seeding.

Whether this increase in infection with the advance in seasonal growth was due to increased plant susceptibility, to accumulated inoculum, or to a

TABLE 1.—*Relation of age of tomato plants to infection by Alternaria solani. Mean readings of infection per (composite) sample lot of 25 tomato seedlings. Harvested at different dates from each of three separate plantings*[a] *in the years shown, and grown for 14 to 18 days as transplants*

1939

Plantings	Harvest dates					Required difference for significance[d]
	5/4	5/8	5/16	5/23	5/30	
1	21.4[b]	21.4	21.4	.....	.....	3.8
2	.....	13.0	19.6	20.8	.....	
3	.....	.....	14.6	16.6	15.0	

1940

Plantings	Harvest dates					Required difference for significance[d]
	5/9	5/14	5/20	5/26	6/1	
1	17.4[c]	56.4	50.6	.....	.....	31.2
2	.....	35.2	31.0	48.8	.....	
3	.....	.....	23.2	39.2	73.4	

1941

Plantings	Harvest dates					Required difference for significance[d]
	4/28	5/7	5/17	5/24	5/31	
1	5.4[c]	2.8	57.2	.....	.....	56.6
2	.....	15.0	44.8	163.4	.....	
3	.....	.....	32.6	97.8	91.4	

1942

Plantings	Harvest dates						Required difference for significance[d]
	5/4	5/7	5/14	5/16	5/19	5/26	
1	17.6[c]	.....	49.2	.....	27.6	.....	21.4
2	.....	6.2	.....	5.8	.....	42.2	
3	.....	.....	17.6	.....	23.0	62.0	

[a] Plantings made in 1939 3/8, 3/18, 3/28; in 1940 and 1941 3/10, 3/20, 3/30; in 1942 3/11, 3/23, 4/2.

[b] Data for 1939 show number of plants infected with leaf spot just before the harvest date.

[c] Number of stem-canker lesions per plant after storage, transplanting, and growth in the field.

[d] At the 1 per cent level.

combination of the two has not been fully determined. Spore traps failed to show an increase in air-borne spores until very late in the season; however, this does not preclude the possibility of an accumulation of spores on individual plants and a consequent rise in infection during the season. Of particular interest is the fact that infection may increase because the tomato plants have become more susceptible to *Alternaria solani* infection through a lowering of the nutritional level. This is suggested by data compiled in 1940–41 and shown in table 2 where equal lots of plants that had received 1, 2, and 3 applications, respectively, of a 4–8–4 fertilizer at 10-day intervals

were inoculated with a spore suspension of *Alternaria solani*. Following inoculation, the plants were stored for 48 hours at temperatures of 70 to 80° F., then transplanted to soil flats in the greenhouse. After growth periods of from 8 to 14 days disease readings were made. It is apparent from these data that the plants became more susceptible as the amount of available plant food was lowered.

TABLE 2.—*Means of Alternaria solani stem lesions or leaf spots on composite sample lots of 50 tomato seedlings grown in soil flats receiving different numbers of applications of a 4–8–4 fertilizer prior to inoculation, storage, and transplanting*

Year	Experiment number	One application	Two applications	Three applications	Required difference for significance[a]
1940	1	41.3[b]	13.6	5.3	3.6
1940	2	216.6[b]	158.3	150.7	6.1
1940	3	122.8[c]	68.5	31.0	5.6
1941	4	320.0[b]	340.2	192.2	9.8
1941	5	137.8[b]	115.1	17.5	5.9

[a] At the 1 per cent level.
[b] Stem canker.
[c] Leaf spot.

The average soils in the South that are normally used for seedling production are fairly low in plant nutrients; also, fertilizer applications on these soils are held to a minimum in order to prevent too succulent growth during periods of excessive rain and when the seedlings reach shipping size. In the light of the data presented in table 2, it is possible that a part of the increased field infection is a result of these conditions, in causing a lowered physical vigor of the tomato seedlings as they grow older.

The immediate application of these findings to the requirements of the various canning areas of the North becomes strikingly evident. Due to such factors as varieties, weather conditions in the growing areas, and transportation, buyers quite often wish to draw plants from a given field for several weeks, thus causing them to become over-aged and more susceptible to infection. The above results indicate that this is a very undesirable practice.

INFLUENCE OF WILTING ON SUSCEPTIBILITY TO INFECTION BY ALTERNARIA SOLANI

Because of the large volume of tomato seedlings the average commercial grower is forced to handle during the very short spring shipping season, instances of poor handling and packing become commonplace in most sheds. One of the most serious of these errors is that of allowing excessive wilting of plants that are not packed promptly after pulling. The average elapsed time between pulling and final packing is about 8 hours, although periods of 24 hours are not unusual. With fairly high temperatures and medium relative humidity prevailing in the packing sheds at all times, it is obvious that there is a considerable physical deterioration from evaporation and respiration during this period, although the plants freshen again after pack-

ing. Observations of disease incidence due to certain types of plant injuries (3) suggested the possibility of a relationship between plant wilting and infection by *Alternaria solani*. Accordingly, a series of experiments on this point was started in the spring of 1939 and continued through 1942.

Tomato seedlings of uniform size and vigor were pulled in bundles of 50 and treated as follows: 5 bundles were packed immediately with wet moss and wrapped with paper; a second lot of 5 bundles was allowed to remain on the table in the packing shed for 6 hours, after which the bundles were packed and wrapped as above; a third lot of 5 bundles was allowed to remain in the shed for 12 hours before packing; and a fourth lot of 5 bundles was held for 24 hours before being packed. After packing, all bundles were placed immediately in baskets and held in storage at room temperature until each lot had been stored for 48 hours. Following this period, each lot of plants was transplanted to the field. The different lots were planted in blocks of randomized rows, the blocks being replicated 5 times. No artificial inoculations were used, the inoculum coming entirely from such natural sources as are present in the average tomato field. After growing for 14 to 18 days, each replicate was pulled separately, washed, and the total number of stem lesions recorded for the 250 plants of each treatment. This procedure was followed in 2 experiments conducted in 1939, and in additional tests in 1940 and 1942.

The data for the 4 experiments are given in table 3 and also are shown

TABLE 3.—*Means of Alternaria solani stem lesion readings in four tomato experiments where samples of 250 seedlings in five replicates were subjected to varying periods of wilting prior to packing and storage*

Date	Mean number stem cankers per 250 seedlings				Required difference for significance[a]
	No wilting	6-hour wilting	12-hour wilting	24-hour wilting	
June 1939	201.8	342.0	404.6	474.0	8.7
Oct. 1939	115.6	178.6	288.2	313.8	5.9
May 1940	278.8	369.2	429.3	473.7	8.5
June 1942	188.4	233.6	313.8	299.6	10.4

[a] At the 1 per cent level.

graphically in figure 1. It is apparent that tomato seedling susceptibility increased as the period of wilting increased. The amount of disease increased significantly from the short- to the long-time periods in all of these experiments. From the standpoint of both the grower and the buyer the practical importance of these findings is apparent.

In order to further verify the role of wilting in these experiments, 2 other tests were made in which the plants were pulled and reset immediately in medium dry soil in plots replicated as described above, but the plants were watered at setting as follows: first lot, no water; second lot, 50 cc. per plant; third lot, 100 cc. per plant; fourth lot, 200 cc. per plant; fifth lot, 500 cc.

per plant. An appreciable amount of wilting occurred on the lots receiving no water, 50 cc., and 100 cc., and progressively less amounts on the lots receiving larger quantities of water. After periods of from 14 to 16 days, each replicate was pulled separately, washed, and the total number of stem lesions recorded. Data for the 2 experiments are shown in table 4. From

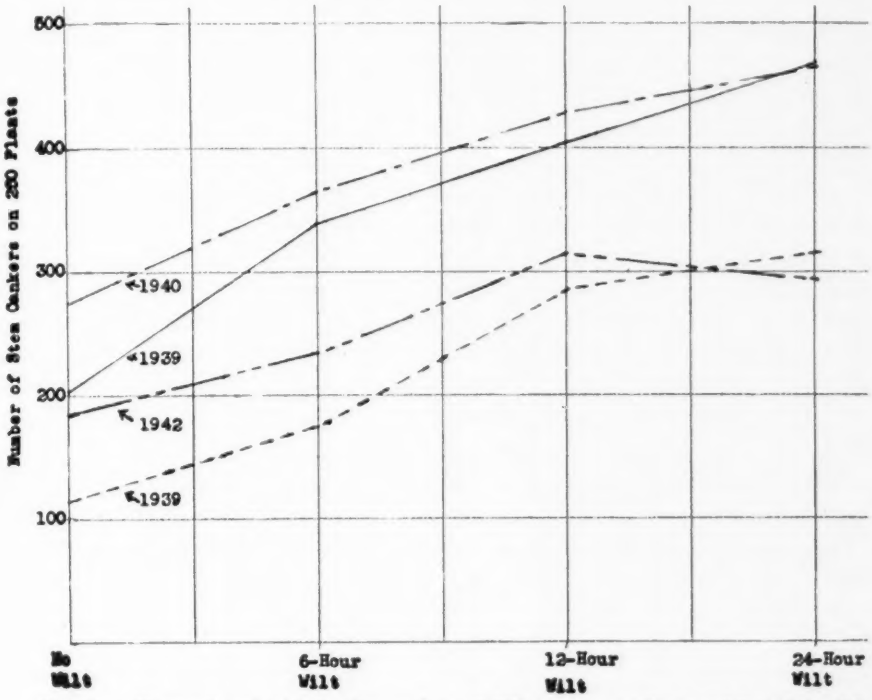

FIG. 1. *Alternaria solani* infection on lots of 250 tomato seedlings subjected to different periods of wilting before packing and storage.

TABLE 4.—*Means of Alternaria solani stem lesion readings for 150 plants in tomato seedling experiments where varying amounts of water were applied to the plants at time of transplanting*

Year	Experiment	Cc. of water per plant					Required difference for significance[a]
		No water	50	100	200	500	
1939	1	214.0	118.4	78.0	80.2	40.6	6.7
1939	2	98.3	98.1	89.6	72.5	35.5	5.1

[a] At the 1 per cent level.

these data it is evident that stem-canker development decreased as the amount of water per plant was increased. Apparently, high disease incidence in these experiments was associated with wilting in the same manner as was shown in the first series of wilt tests.

THE RELATION OF LENGTH OF STORAGE TO INFECTION BY ALTERNARIA SOLANI

The main portion of tomato plants produced in the South go to the canning areas of the North, and consequently require a shipping period of 24 to 60 hours. In many instances plants cannot be transplanted for a day or two after they are received at their destination. Since rather high temperature and high humidity exist in the baskets or crates in which the seedlings are packed, any prolonged lapse of time between time of shipment and final transplanting is likely to influence disease development. During the course of nutrient studies on tomato seedlings during the seasons of 1939, 1941, and 1942, a number of storage tests were conducted in which it was possible to make disease comparisons between lots of plants subjected to 2-day and 4-day storage periods. Lots of 5 bundles of 25 plants were packed according to commercial practice, then stored at room temperature. At the end of a 2-day period one lot was removed and transplanted to the field and another was similarly treated at the end of a 4-day period. All plantings were allowed to grow for periods of 14 to 18 days, after which each lot was pulled separately, washed, and the stem lesions recorded. The combined data for 7 of these storage tests conducted at Tifton, Ga., are shown in table 5.

TABLE 5.—*Mean number of Alternaria solani stem lesions found on lots of 50 tomato seedlings subjected to different periods of storage prior to transplanting*

Date	Number replicates	2-day storage	4-day storage
June 1939	6	157.5 ± 14.3[a]	229.1 ± 2.9
June 1939	5	43.0 ± 7.4	67.0 ± 6.6
June 1939	5	127.2 ± 13.4	259.2 ± 7.8
June 1941	5[b]	128.4 ± 17.3	213.6 ± 20.0
June 1941	5[b]	371.6 ± 34.8	691.2 ± 16.1
June 1942	5	1368.6 ± 116.3	1601.0 ± 166.8
June 1942	5	708.6 ± 36.2	1212.8 ± 126.0
Totals	.....	407.8 ± 70.2	599.9 ± 64.3

[a] Standard error of the mean.
[b] 3-day and 6-day storage.

These data indicate that infection by *Alternaria solani* increased significantly when the storage was extended from 2 to 4 days.

In 1938 a test was conducted with locally grown tomato seedlings that were inoculated with a suspension of *Alternaria solani* spores, packed in peat moss, then stored at room temperature (70° to 75° F.). After the desired periods of storage, the plants were transplanted to greenhouse benches. Stem-lesion data (Table 6) were recorded after 7 days.

Storage tests similar to the above also were conducted in Indiana in 1939, 1940, and 1942. Tomato seedlings grown in Georgia were packed as previously described and shipped by express to Lafayette, Ind. Upon arrival one lot was set in the field immediately and another held in storage for 2 or 3 days before setting. Since the normal shipping time between

Georgia and Indiana is 2 days, plants set immediately upon arrival were considered as having had 2 days of storage. The number of stem lesions or leaf spots was recorded after growth periods that varied in the different years from 11 to 27 days after setting. The data from these experiments are also given in table 6.

TABLE 6.—*Mean number of Alternaria solani stem lesions or leaf spots found on lots of 16 tomato seedlings subject to different periods of storage prior to transplanting in Indiana*

Date	Short storage ave. number per plant	Long storage ave. number per plant	Required difference for significance[e]
May 1938[a]	0.3	2.4	0.8
June 1939[b]	0.1	1.6	0.6
June 1940[c]	0.3	0.2	....[f]
June 1942[d]	0.9	1.5	0.7[f]

[a] Stem lesions after 1 and 3 days storage.
[b] Leaf spots after 2 and 4 days storage.
[c] Stem lesions after 3 and 6 days storage.
[d] Stem lesions after 2 and 5 days storage.
[e] At the 1 per cent level.
[f] F value not significant.

From the data shown in table 6, it is apparent that in 1938 and 1939 *Alternaria solani* infection increased on the tomato plants stored for the longer period; but in 1940 and 1942 there was no significant difference between storage periods. It should be noted, however, that 1940 and 1942 were seasons in which disease incidence was very low on all seedlings shipped from Georgia.

DISCUSSION

The commercial production of tomato seedlings in the South and the problem of transporting them to northern canning areas is a complicated agricultural enterprise. Consideration must be given to such factors as plant nutrition, horticultural practices, weather conditions, diseases, and transportation. Whether acting individually or collectively, these several influences can affect both the general production problem and the specific problem of infection by *Alternaria solani*. Recognition of the interrelationships of these several factors is necessary in attempting to develop a system of control for this disease. The fact that increasing age of plants was found associated with increasing susceptibility to infection by *Alternaria solani*, as shown by the data in table 1, is important as only one factor among the several that are involved in the whole disease problem. Some canning areas of the North demand plants early in the spring, while others want them late. By making interval plantings in the South to meet the requirements of the respective areas of the North one phase of disease control that appears to have appreciable merit may be instituted.

While some undesirable circumstances involved in tomato plant production can be entirely removed, others can be modified or improved only to a degree. Since plant wilting cannot be entirely prevented, some damage

from this cause appears unavoidable. However, the fact that prolonged periods of wilting increased the infection by *Alternaria solani* indicates that appreciable reductions in disease losses may be possible through adjustments of field and packing-shed practices so that wilting periods will be reduced to the shortest possible time.

Time lapse in tomato-plant transportation between any two points is fairly well fixed by train schedules; consequently, holding periods for any particular shipment cannot be reduced below this minimum. However, storage of plants for additional periods of time after arrival in the North appears, on the basis of the data in tables 5 and 6, to be an undesirable practice. *Alternaria solani* is known to develop rather rapidly under conditions of high humidity and temperature and, since the average tomato plant shipment is subjected to such an environment, it is reasonable to expect increased damage from unduly extended shipping and storage periods, particularly since the present experiments indicate that during most seasons there is sufficient inoculum on the tomato plants for heavy natural infection. Experiments under way at the present time in cooperation with E. V. Miller of the Methods of Handling, Transportation and Storage section of the Division of Fruit and Vegetable Crops and Diseases, suggests that plants may be shipped successfully in refrigerator cars where optimum temperatures can be maintained by the limited use of ice in the bunkers, thus eliminating much of this damage.

CONCLUSIONS

Amount of infection of tomato seedlings by *Alternaria solani* increases with age of the seedlings.

Susceptibility of tomato seedlings to infection by *Alternaria solani* increases as periods of wilting are increased.

Alternaria solani infection of tomato seedlings increases as the period of storage is lengthened.

Substantial reductions in losses or damage of southern-grown tomato seedlings, from *Alternaria solani*, can be brought about by avoiding too early planting or use of over-age plants, by prompt and careful packing to avoid wilting and mechanical injury, and by transporting and transplanting with a minimum of delay.

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EFFECT OF MINERAL NUTRITION ON FLOWER PRODUCTION OF OWN-ROOTED ROSES AND THE INCIDENCE OF BLACK-SPOT

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In a nutrition experiment comparing grafted, budded, and own-rooted roses it was found that plants of the same variety receiving solutions of various composition responded differently to black-spot infection. This experiment was a project of the research department, Yoder Bros., Barberton, Ohio. The findings with own-rooted roses grown under greenhouse conditions and the results of studies on outdoor roses will be included in this paper. The latter were obtained from the writer's rose garden at Doylestown, Ohio.

THE EFFECT OF MINERAL NUTRITION ON OWN-ROOTED ROSES GROWN UNDER GREENHOUSE CONDITIONS

The experiment was planned to study the effect of nutrient solutions of different composition on flower production, blindness, and bullhead formation. Gravel culture units containing Haydite as the growing medium were used. Equal numbers of young rooted rose cuttings were planted on November 18, 1938, in 6 independent gravel units. The plant material consisted of the following varieties: Briarcliff, Better Times, Johanna Hill, Ellen, and Hollywood. In the course of the investigations 4 gravel units were discontinued in April, 1939, leaving the 2 units for flower production. These units were in the center of the greenhouse and similarly located in regard to its construction.

The following nutrient solutions were applied to the plants:

Withrow E–2					
Salts	K_2SO_4	$CaH_4(PO_4)_2 \cdot H_2O$	$Ca(NO_3)_2$	NH_4NO_3	$MgSO_4 \cdot 7H_2O$
Purity of salts	95 per cent	Monsanto	82 per cent	90 per cent	
Millimolar concentration	6.0	0.5	4.0	4.0	0.5
Mader R–1					
Salts	KNO_3	$CaH_4(PO_4)_2 \cdot H_2O$	$Ca(NO_3)_2$	NH_4NO_3	$MgSO_4 \cdot 7H_2O$
Purity of salts	90 per cent	Monsanto	82 per cent	90 per cent	
Millimolar concentration	9.0	2.0	5.3	8.0	4.0

Solution Withrow E–2 was recommended by R. B. Withrow of Purdue University, Lafayette, Indiana, as "a solution for use during bright weather" and suitable for conditions under which the experiments were planned. Solution Mader R–1 was selected from a series of solutions under investigation, and was found most suitable for rose production under the conditions of these experiments.

The solutions were changed every 3 weeks, and the culture medium completely and repeatedly flushed with water in order to remove any excess salt.

The pH of the solutions was kept at 6.5 by daily adjustment. To these solutions the following minor elements were added: iron, 7.5 p.p.m. weekly; boron 0.5 p.p.m.; copper and zinc 0.05 p.p.m. and manganese 1 p.p.m. every 3 weeks. The units were subirrigated and 100 gal. of solution to 66 square feet of bench space were used. A 4-hour interval of automatic subirrigation was maintained throughout the experiment. In order to prevent the growth of algae on the surface of the medium the solution was pumped to within 1 inch of the top layer only. The young plants were constantly pinched (soft-pinched) until May 15, 1939, in order to build up the plant structure. Starting July 1, 1939, a production record of flowers and bullheads was kept (Table 1).

TABLE 1.—*Cut-flower and bullhead record July 1 to Nov. 30, 1939*

Variety	Number of plants	Cut-flowers		Bullheads	
		Total number	Average per plant	Total number	Average per plant
Solution Withrow E–2					
Briarcliff	24	194	8.08	15	0.63
Better Times	24	201	8.38	11	0.46
Johanna Hill	12	120	10.00	1	0.08
Ellen	12	78	6.50	1	0.08
Hollywood	18	78	4.33	2	0.11
Solution Mader R–1					
Briarcliff	24	340	14.16	63	2.63
Better Times	24	246	10.25	21	0.88
Johanna Hill	12	199	16.58	2	0.17
Ellen	12	188	15.67	8	0.67
Hollywood	18	200	11.11	9	0.50

The difference in the effect of the 2 nutrient solutions is evident. Solution Mader R–1 supported a more vigorous plant growth and flower production than solution Withrow E–2. Plants under the latter treatment produced a type of growth tending toward blindness which, in part, may explain the lower flower production.

Plants treated with Mader R–1 tended toward bullhead formation. The term "bullhead" designates a type of growth resulting in partly developed flowers. The bullhead usually is formed on a vigorous cane, and in most cases is associated with an over-fertilization with nitrogen, particularly NH_4NO_3. Although its nature is but partly understood, this abnormal physiological behavior in the rose plant seems related to its reaction to black-spot. Apparently, the more vigorous the plant the less prevalent is black-spot.

In the early part of August, 1939, a heavy storm deranged the ventilating system of the greenhouse. The accompanying rain kept the foliage of the plants wet for more than 6 hours. About 10 days later the plants appeared as if they had been subjected to mass inoculation with the black-spot fungus. Although the infection occurred by chance, the response of the plants under the 2 solution treatments was of interest. The own-rooted ones were selected

because they had been subjected to these 2 nutrient solutions throughout their development. No counts were taken on grafted and budded plants included in this experiment.

Premature leaf drop (a result of black-spot infection and of syringing for red spider) did not permit further counts of diseased leaflets. Counts were made from 4 plants of each variety, selected at random (Table 2).

TABLE 2.—*Total number of healthy and diseased leaflets, as well as the number of black-spot lesions*

(Figures are averages per plant)

Variety	Healthy leaflets	Leaflets showing lesions	Total number of lesions
Solution Withrow E–2			
Briarcliff	287	131	590
Better Times	352	136	612
Johanna Hill	293	193	885
Ellen	382	185	710
Hollywood	294	158	647
Solution Mader R–1			
Briarcliff	373	29	51
Better Times	441	22	41
Johanna Hill	354	53	86
Ellen	517	29	54
Hollywood	371	24	53

The difference in the number of diseased leaflets and black-spot lesions for the 2 sets of plants are striking. For some reason not yet clearly understood, the per cent of infection on the leaflets of the Mader plants was considerably lower.

It was noted that the black-spot lesions on the Withrow-solution plants exhibited a different pattern from those of the Mader plants. They were grayish, and their radiating margins gave them a feathery appearance. After a certain length of time they became more or less circular, black (necrotic) lesions, ranging from 1 to 2 cm. in diameter. Only a few lesions of this type could be found on the Mader plants, most of the lesions being small, well-defined, circular black spots. Some of these appeared like mere pin points. These lesions, particularly the small ones, suggest that infection occurred, but the progress of the disease was soon checked by the physiological state of the plants. Probably some of the smaller lesions were overlooked in the counts, hence the apparent difference in the number of lesions recorded on the two sets of plants.

Due to the nature of the experiment, no definite conclusions can be drawn as to the effect of mineral nutrition on the resistance-susceptibility of roses to black-spot. The results obtained are only suggestive. Although black-spot infection was incidental, the difference in its severity in the two sets of plants was striking. Plants with a tendency to bullhead formation, and exhibiting the most vigorous growth, together with the highest production of flowers, had the least black-spot (solution Mader R–1). These findings

confirm those of Gregory[1] who states "that many rose growers in Indiana believe that keeping the plant growing vigorously and steadily by the use of fertilizer and copious watering is an important factor in black-spot control."

Apparently a solution like Withrow's E–2, if used under conditions similar to those under which these experiments were conducted, helps to determine why the resistance of the plant is not being built up.

As nutrient solutions in gravel culture units are kept in a closed system for 3 to 4 weeks, and as there is an unequal absorption of the nutrients by the plant, the remaining salts and their combinations probably influence its physiological balance, which in turn governs the intake of ions and thus influences the type of plant growth. This assumption is supported by analyses of the two sets of plants. Differences in protein composition, as well as rate of formation, are indicated (incomplete data). It is suggested that the difference in the reaction of the rose to black-spot will be determined by the ability of the plant to obtain from the solution the necessary nutrients.

THE EFFECT OF MINERAL NUTRITION ON OUTDOOR ROSES

The question naturally arises as to whether or not it is possible to extend the findings of the greenhouse studies to outdoor roses, particularly in regard to mineral nutrition and control of black-spot.

In the spring of 1942 a trial experiment on feeding outdoor roses entirely with inorganic salts was conducted. The garden is located at Doylestown, Ohio, and a description of its site was given by Massey.[2] The soil comprising the rose beds was a tile-drained mixture of one-half subsoil (loam) and one-half field soil (sandy loam). In the spring of 1942, 540 plants of the varieties Briarcliff, Johanna Hill, Better Times, Pink Delight, Yellow Gloria and Chieftan were set out.

The Outdoor Rose Solution

Shortly after the indoor black-spot studies (1938–1939), the solution Mader R–1 was modified. Additional rose-nutrition experiments (unpublished) showed that by reducing the total nitrogen, particularly NH_4NO_3 in the solution, bullhead formation dropped and the total flower production increased. Unavailability of $Ca(NO_3)_2$ made it necessary to change to KNO_3 as the main source of nitrogen. The composition of the solution is as follows:

Mader R–2

Salts	KNO_3	$CaH_4(PO_4)_2 \cdot H_2O$	$CaSO_4$	$(NH_4)2SO_4$	$MgSO_4 \cdot 7H_2O$
Purity of salts	95 per cent	Monsanto		95 per cent	
Millimolar concentration	15.5	2.0	3.5	1.4	3.5

[1] Gregory, C. T. Fertility and the black-spot of roses. Better Crops with Plant Food 173: 17, 56–57. 1931.

[2] Massey, L. M. Diseases as influenced by garden site. Amer. Rose Magazine 4: 293–294. 1942.

The same concentration was used for feeding outdoor roses, but instead of using 100 gallons of such solution for 66 square feet bench space, as for indoor roses, 100 gallons were applied to 528 square feet of outdoor bed space. By following this procedure, approximately the same nutrient level was maintained for outdoor roses in soil as for indoor roses in gravel. The feeding of outdoor roses was bi-weekly until August 1, 1942, followed by monthly feeding until September 30, 1942. No organic material or humus was added to the soil in this experiment. Water was supplied when necessary by flooding the beds.

Although the weather of the 1942 season favored black-spot, it was observed that roses under treatment remained practically free. In frequent examinations the 540 plants showed only 157 leaves infected with black-spot. The lesions revealed the same characteristic patterns as those described for indoor plants under solution treatment Mader R–1, that is, they had well-defined margins, were small, and soon turned black. Undoubtedly the selection of a garden site having a lower dew point than its surrounding area was a factor in keeping the infection low. The type of lesions present indicated that the solution used must have been responsible to some extent for the increased resistance to black-spot fungus. The roses in this outdoor experiment exhibited an excellent vigorous growth and were prolific in flower production. Further tests are needed to differentiate between the effect of garden sites and mineral nutrition on the control of black-spot.

SUMMARY

Data here presented indicate that rose plants, fed with nutrient solutions of various compositions, responded differently to black-spot infections.

Plants with a penchant for bullhead formation and exhibiting the most vigorous growth, together with the highest flower production, had the least black-spot; whereas plants tending toward blindness, together with the lowest flower production, showed the most. There is an apparent difference in pattern of black-spot lesion. Those on the leaves of the former plants were smaller, well-defined circular black spots, while those of the latter were larger, bore radiating margins (giving them a feathery appearance), grayish, and turning black only after a certain length of time.

An attempt was made to extend the findings of the greenhouse studies to outdoor roses, particularly in regard to mineral nutrition and control of black-spot.

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ERADICANT ACTION OF FUNGICIDES ON SPORES ON LIVING PLANTS

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INTRODUCTION

Methods of plant disease control by fungicides have been designed heretofore to protect plants against fungous infection by applying a deposit of fungicide to the plant surfaces before spores are present. The resulting control has been considered to be a measure of the protective value of the material.

In the designing of control methods on living plants little attention has been paid to evaluating the effect of fungicides on the spores of fungi that are present when the fungicide applications are made.

The present study is not a test of fungicides as such but concerns itself only with the eradicant action of fungicides on fungus spores present on living plants and the measurement of this action in terms of spore mortality and its relation to subsequent control of the fungus. For the purposes of this paper the term eradicant refers to that action of a fungicide that renders fungus spores inactive and inhibits their germination in the infection court, so that they are incapable of producing infection.

A review of the literature on disease control by fungicides showed a wide range of reports on the eradicant action of fungicides on disease organisms; the majority of the reports deal with apple scab and seed treatment. From the literature it is evident that the eradicant action of fungicides has long been recognized, and in some control programs has been put to good use. The literature reports, for the purposes of this review, will be confined to those dealing with apple scab in the conidial stage in an active state.

Apple Scab. In 1922 Doran[3] reported that conidia of *Venturia inaequalis* are killed when they are hit by fresh lime-sulphur, but that this antiseptic action of lime-sulphur is not lasting, since the character of the fungicide changes on weathering. Hamilton[4] reported in 1932 that sprays containing liquid lime-sulphur may have a very considerable immediate fungicidal effect at the time of application, before the spray becomes dried and decomposed, which the wettable sulphurs do not have. Later, regarding scab control, Hamilton[5] classified spray materials on the market as those

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[4] Hamilton, J. M. Recent investigations on the control of apple scab in the Hudson Valley. N. Y. (Geneva) Agr. Exp. Stat. Bull. 604. 1935.

[5] Hamilton, J. M. Studies on apple scab and spray materials for its control in the Hudson Valley. N. Y. (Geneva) Agr. Exp. Stat. Tech. Bull. 227. 1935.

that are both protective and eradicative, such as lime-sulphur and, to a lesser extent, dry lime-sulphur, and those that are generally merely protective, such as wettable sulphurs, sulphur dusts, and copper sprays.

METHODS AND MATERIALS

The general method was to spray the fungicide on infected plants, take spores from the sprayed lesions, determine the spore mortality and relate these mortality data to control. Spore mortality was determined from the percentage that failed to germinate when removed in a large volume of water. The spores may not be killed, only inhibited, and all the adhering fungicide probably did not come off in the water. However, from the standpoint of preventing infection, it matters little whether the spore was inactivated before or after it was washed or splashed to a new locality. *Uromyces caryophylinus* on carnation and *Venturia inaequalis* on apple were the fungi and plants used in this experiment.

Various organic and inorganic fungicides were used. The organic materials were tetrachloro-para-benzoquinone (Spergon), tetramethyl thiuram disulphide[6] (Japanese Beetle Spray), ferric dimethyl dithiocarbamate (Fermate), mercaptobenzothiazole (Captax), and dinitro-o-cresol dye (Elgetol). The inorganic materials were liquid lime-sulphur, wettable sulphur (Mike), copper oxychloride (Compound A), yellow cuprous oxide (Yellow Cuprocide), and Bordeaux mixture.

Carnation Rust

Greenhouse Spraying. Various materials were sprayed on infected plants in a commercial greenhouse to measure their eradicant effect on the spores present. Four applications were made: Jan. 29, Feb. 6, 20, and March 13. The viability of the spores on sprayed leaves was determined at various times during the course of the experiment. Spore suspensions

TABLE 1.—*Eradicant effect of various spray materials on the urediospores of carnation rust and on the control of new leaf infections*

Material	Per cent concentration	Per cent spore germination			New infections[b]
		2 hr.	17 days	35 days[a]	35 days
Check	.....	60	75	63	20.6
TMTD	0.25	0	0	6	0.9
Spergon	0.25	18	11	8	1.7
Fermate	0.25	0	23	22	1.8
Yellow Cuprocide	0.25	16	43	.....	7.8
Captax	0.25	37	69	62	6.4
Mike Sulphur	0.75	52	61	61	6.1

[a] Time of interval after fourth spray application.
[b] Above the level of infection when spraying started. 1000 leaves counted.

[6] Hereinafter designated as TMTD, for brevity.

for germination studies were prepared as follows: 30 leaves of comparable age were taken from the plants in each treatment; spores were then taken from pustules of comparable size on each of the 30 leaves for each treatment, the final concentration of spores in double-distilled water was adjusted to 5000 to 1 cc.; drops of the spore suspensions were placed on glass slides, and incubated in moist chambers at 20° C. over night; germination counts were then made, counting 600 spores for each treatment (Table 1).

Thirty-five days after the last spray application, counts were made on the number of infected leaves in each treatment above the level of infection when spraying began (Table 1).

The data on spore germination in table 1 give a measure of the direct action of the fungicides on the spores and show also that there is a direct relation between mortality of the spores and amount of new infection.

Apple Scab

A field test was made to determine the eradicant action of various fungicides on the conidia of *Venturia inaequalis*, and to measure the action in terms of control. This test was made in such a manner that the eradicant action as measured by spore inhibition could be related to amount of infection on new leaves produced after spraying was done.

Late in April, over-wintered apple leaves were placed under young (2-year-old) McIntosh trees. Ascospore discharge occurred on May 15, 16. No further ascospore discharge occurred and scab lesions appeared on the leaves by May 27. The infections were allowed to develop unchecked for 9 days. On June 5 the trees were sprayed with various fungicides, using 2 dosages of each material to give varying levels of spore inhibition. Each dosage was applied to 4 replicated blocks of 5 trees each. On June 16, 11 days after spraying, the effect of the fungicides on mortality of the conidia was determined.

Four sprayed infected leaves were collected from each of the 5 trees in the 4 replicate blocks, making a total of 80 leaves for each dosage, from which 40 leaves were selected at random for the working sample. A $\frac{3}{16}$-inch disc was cut out of a scab lesion on each of the 40 leaves and these discs were shaken in distilled water to make a spore suspension, drops of which were incubated on glass slides for 48 hours at room temperature, after which germination counts were made (Table 2).

A record was made on July 21 (46 days after spraying) of the amount and severity of infection on the new leaves produced after spraying was done (Table 2). The new leaves were separated into 2 groups: those on side and those on top branches. This was done to determine if the possible washing by rain of spray materials from the sprayed leaves onto the unsprayed leaves had any significant effect on the amount of scab on the unsprayed leaves.

Table 2 shows that the data on infection of new leaves on both side and

top branches were in general agreement with the data on spore inhibition; the trees sprayed with materials causing the highest spore mortality had the least amount of infection on the new growth. The data show also that there was more infection on the new leaves on the side branches than on the tops of the trees, whatever the materials used. From this it is concluded that washing of spray by rain onto unsprayed foliage had very little if any effect on control, and that the amount of infection on unsprayed leaves is a direct measure of the eradicant value of the sprays as no protective action was possible.

TABLE 2.—*Effect of various fungicides on the germination of apple scab conidia and on the amount and severity of scab infection on new leaves produced after spraying*

Material[a]	Concentration	Spore[b] germination	Infection on new leaves[c]			
			Side branches		Top branches	
			Leaves scabby	Severity	Leaves scabby	Severity
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Check	.....	64.0	74.8	41.0	30.2	11.1
TMTD	0.2500	1.5	48.0	21.0	19.0	6.8
	0.0625	2.0	56.5	27.5	18.5	6.0
Spergon	0.2500	16.0	61.2	27.5	21.5	7.3
	0.0625	66.0	67.2	39.5	27.0	11.3
Fermate	0.2500	0.0	39.8	18.4	15.3	4.3
	0.0625	0.0	56.1	24.0	19.7	5.9
Mike Sulphur	0.2500	2.5	58.0	23.8	20.5	7.0
	0.0625	46.0	75.5	31.8	36.5	11.4
Lime-sulphur	0.2500	29.5	56.7	26.0	15.5	4.9
	0.0625	57.0	77.0	24.8	25.4	8.8
Yellow Cuprocide	0.2500	0.0	16.0	3.5	7.5	1.9
	0.0625	0.0	17.5	6.3	8.0	2.6
Elgetol	0.2500	19.0	64.9	33.3	28.7	8.9
	0.0625	74.0	69.0	20.3	32.3	10.9

[a] Materials applied on June 5.
[b] 11 days after spraying.
[c] 46 days after spraying.

The material that gave the best results, Yellow Cuprocide, was also the most injurious to the foliage; but, as we were not interested in the fungicide as such, the injury is not considered important in this experiment.

It should be mentioned here that in this experiment the materials were all used at the same percentage concentrations. For this reason lime-sulphur and Mike Sulphur were used at strengths below those recommended for use in the field. Despite this, both materials still had considerable eradicant action at the concentrations used.

DISCUSSION

These experiments have demonstrated the eradicant action of fungicides on spores and sporulating hyphae of fungi on living plants and have pro-

vided techniques for measuring such action. The apple-scab experiment was so designed as to eliminate the influence of protective action on the results.

The carnation-rust experiments provide a yardstick for measuring eradicant action on the spores themselves and on sporulating hyphae. It will be noted that the most effective materials inhibited approximately the same proportion of spores in the population at the end of 35 days as at the end of 2 hours. This indicates an eradicant action on the spore-producing hyphae themselves. If they had continued actively to produce new spores the percentage of viable spores should have increased in time. The demonstration of this latter action gives a measure of eradicant action not obtained by laboratory techniques. The relation between spore mortality and control is strikingly close, even though eradicant and protective values are not clearly separated in this experiment.

The apple-scab experiment provides 2 measures of the eradicant action of the materials on the spores: 1. Spore inhibition and 2. Prevention of new infection on unsprayed tissue. The latter was accomplished by taking the data on control from leaves that grew after the spraying and, consequently, had no protective spray. Inasmuch as the spread of conidia of *Venturia inaequalis* is very limited, being confined essentially to the individual tree, infection on the new leaves must of necessity be caused by spores produced on that same tree. It will be noted that high spore mortality resulted in less scab on the new foliage on both top and side branches. The difference in amount of infection on the unsprayed leaves of top and side branches further corroborates the effect of eradicant action of the materials used. It has been thought that a fungicide sprayed onto tree foliage may be redistributed by rain to uncovered leaves and thus prevent their infection. If so the redistribution would be expected to be more outward and downward than upward. Hence, the lower and outer branches would be better protected than the upper branches and, consequently, less heavily infected, but they were not; in fact, the reverse was true. This indicates that the spores were splashed outward and downward more effectively than the fungicide and, hence, the increase of infection on the lower and side branches. If redistribution of the fungicides were indeed a factor in the results of this experiment, it would also be expected that the materials with the highest coefficient of tenacity would give the least control because they would be splashed around least, but if we compare Yellow Cuprocide with a tenacity coefficient of 0.834 and Mike Sulphur with a tenacity coefficient of 0.35, it is found that the reverse is true; and that the fungicide with the highest tenacity coefficient gave the best control. From these facts we must conclude that on the unsprayed foliage in this experiment the redistribution of fungicides was not a factor in the control of apple scab, and the amount of infection on the unsprayed foliage is related to the eradicant action of the fungicides on the conidia of apple scab present on the foliage at the time of spraying.

SUMMARY

It is shown that a range of fungicides of varied chemical compositions (copper, sulphur, organic) have an eradicant action on the spores of fungi present on living plants when spray applications are made.

The experiments show that control of plant diseases on living plants by fungicides is due to their eradicative action on the fungi present on the plant, as well as their protective value to the plant.

Techniques are described for measuring the eradicant action of fungicides in terms of spore mortality and control.

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A GRAFT TRANSMISSIBLE MOSAIC OF AMERICAN ELM

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(Accepted for publication May 31, 1943)

A disease of American elm (*Ulmus americana* L.), resembling a virosis of the mosaic type and sometimes producing a mild to moderate brooming of branches, was observed in the vicinity of Cleveland, Ohio, in 1927 and was described briefly in 1941.[4]

Since 1927, scattered American elms, showing symptoms somewhat similar to those on the Cleveland trees, have been found throughout a large portion of Ohio and in parts of Kentucky, Michigan, and New Jersey. However, in all of these locations but few diseased trees have been observed.

Symptoms of the disease vary and often occur first on the leaves of a single branch, but eventually the entire crown may become affected. Usually some leaves are normal in size, color, and texture, but others, of the same tree or on the same branch, may be abnormally large and dark-green or small, stiff, mottled, and distorted. The smaller leaves usually develop a distinct yellow-green mottle, sometimes accompanied by rugosity; but diffused, yellow mottling, especially along the midribs, also may occur (Fig. 1). In a few cases yellow or chlorotic ring-spot symptoms have been observed on leaves of normal size.

In advanced stages of elm mosaic some leaf buds may fail to expand, leaving the branches bare of foliage for considerable distances. This gives the tree the appearance of having its leaves in bunches or tufts (Fig. 2). Many times this characteristic makes it possible to detect elm mosaic suspects from a distance. Mild to moderate brooming of branches may or may not be associated with either the yellow-green or diffuse-type mottle (Fig. 3).

No discoloration of bark or wood is produced by elm mosaic, but they frequently are dry, and separate from each other with difficulty. The wood is often brittle.

None of the trees under our observation has been killed by elm mosaic. In some cases there has been a gradual decline in vigor and some branches have died, causing the tree to become unsightly and making its removal necessary.

TRANSMISSION

In June, 1939, roots, bark patches, and branches from 3 elms in Cleveland, Ohio, which had shown mosaic symptoms for a number of years, were grafted to healthy American elms in a nursery at Wooster, Ohio. Leaf

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[4] Swingle, R. U., P. E. Tilford, and Charles F. Irish. A transmissible mosaic of American elm. (Abstract) Phytopath. 31: 22. 1941.

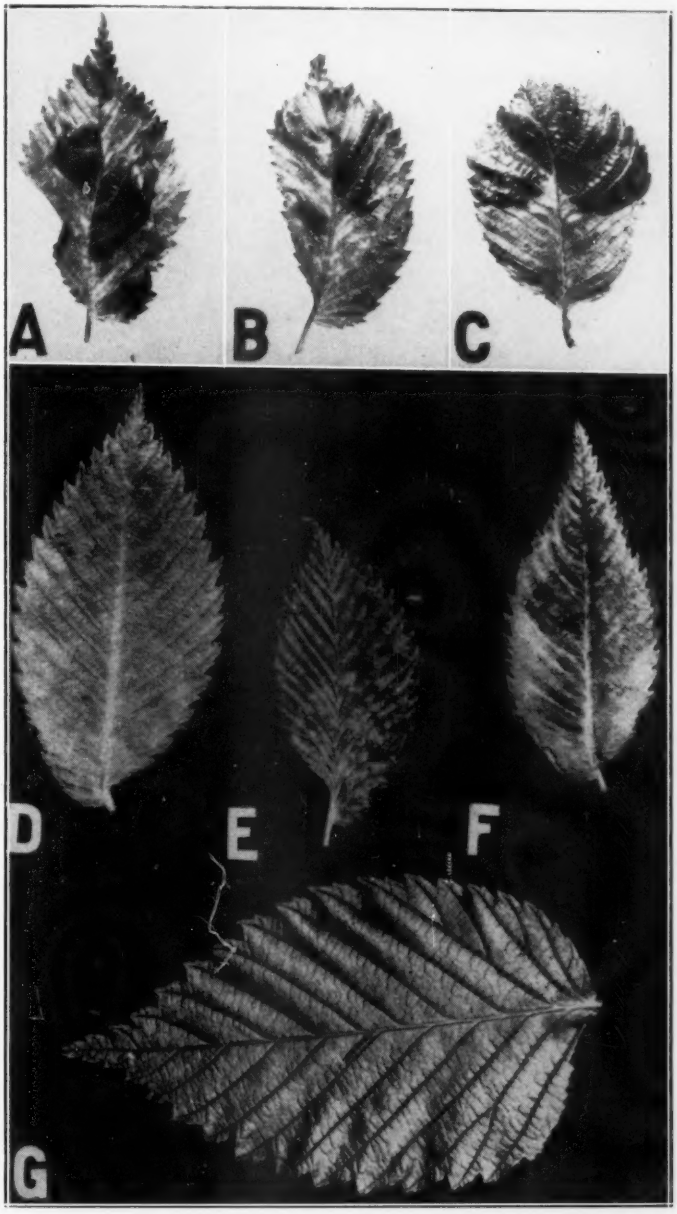

FIG. 1. A to F. Foliar mottling and distortion produced by elm mosaic. G. A healthy American elm leaf.

inoculations were attempted, using expressed sap from diseased leaves with carborundum as an abrasive. The results obtained are given in table 1.

In 1940, bark patches and buds from some of the trees successfully inocu-

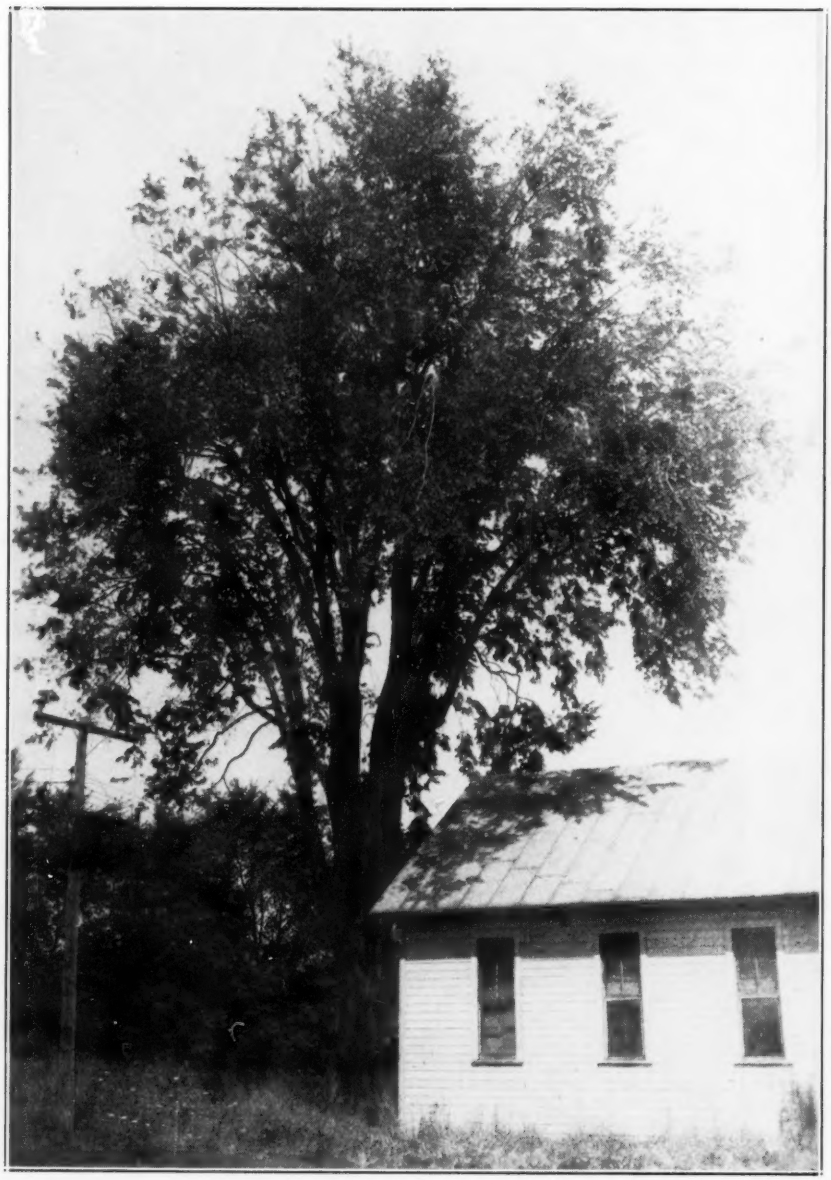

FIG. 2. An American elm affected by mosaic. Many buds fail to expand, producing symptoms of bunched and sparse foliage.

lated in 1939 were transferred to healthy trees in an attempt to retransmit the disease. Five of 6 trees grafted with diseased bark patches and 4 of 6 budded trees developed typical mosaic symptoms the following year.

Other inoculation experiments, initiated in 1940 and 1941, involved grafting over 300 healthy American elms with branch, trunk, and root material from elms with mosaic symptoms, growing in Kentucky, New Jersey,

TABLE 1.—*Transmission experiments, 1939*

Method	No. of trees	No. of trees making graft union	No. of trees showing mosaic symptoms	
			1940	1941
Root splice grafts	6	5	0	0
Bark patch grafts	22	20	19	20
Branch splice grafts	12	0	0	0
Leaf inoculations with expressed sap using carborundum	4	.....	0	0
Checks—grafted with bark patches from healthy elms	3	3	0	0

FIG. 3. Brooming of branches is frequently associated with elm mosaic. Some laterals of the broom are dead.

and several localities of Ohio. So far, none of the trees grafted with root material has developed mosaic symptoms, even though, in most cases, union between stock and scion has occurred. Mosaic symptoms have been pro-

duced consistently, however, by budding and patch grafting with trunk and branch material. Usually, the symptoms produced have been of the yellow-green-mottle type, although diffuse mottle and yellow or chlorotic ring spot symptoms developed occasionally. One of the grafted trees has produced brooms.

How elm mosaic is transmitted under field conditions is not known, but there appears to be some natural spread. In Cleveland and vicinity, where mosaic elms have been under observation since 1927, apparent transmission has been slight. In some instances, healthy trees adjacent to diseased ones, sometimes with their branches intermingling, have remained healthy. In other instances, healthy trees growing near diseased ones have developed mosaic symptoms after a few years.

At Chillicothe, Ohio, 1 mosaic elm was found in 1938 in a group of trees growing in a cemetery. In 1941, 6 additional elms nearby developed typical mosaic symptoms. Later, some of these trees also developed symptoms of elm-phloem necrosis[5] and died soon thereafter.

A few other trees affected by both elm mosaic and elm-phloem necrosis have been found during investigations of the latter disease. In some of these trees, symptoms of phloem necrosis only were evident, but upon grafting healthy trees with bark patches from them, symptoms of both elm mosaic and phloem necrosis were obtained.

CONTROL

No control measures for elm mosaic are known. A number of diseased trees have been fertilized over 2- and 3-year periods by the Aero-Fertil Method, using a 10–6–4 fertilizer. None of the fertilized diseased trees has shown evidence of increased vigor. They have continued to decline, and symptoms of bunched and sparse foliage have been retained.

During the past 15 years several attempts have been made to control the disease in trees showing initial symptoms on a few branches by removing the branches bearing mottled foliage. In all of these cases, however, symptoms have continued to develop on the leaves of the remaining branches.

CONCLUSION

A disease of American elm (*Ulmus americana*), producing mottled foliage and sometimes brooming of branches, has been transmitted by grafting. It has been concluded from symptom and transmission studies that the causal agency is a virus. Means of spread under field conditions and control measures for elm mosaic are not known.

[5] Swingle, Roger U. Phloem necrosis, a virus disease of the American elm. U. S. Dept. Agr. Cir. 640. 8 pp. 1942.

A HITHERTO UNREPORTED DISEASE OF THE WASHINGTON PALM

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(Accepted for publication May 12, 1943)

A previously unreported disease of the Washington palm (*Washingtonia filifera* Wendl.) was first noticed on the campus of the University of Arizona in the late fall of 1942. Investigation disclosed that the malady was widely distributed throughout the city of Tucson. Beyond that, its prevalence is not known.

SYMPTOMS

The first symptom of the disease is a pin-head sized or even smaller, water-soaked spot (Fig. 1, *B*, *a*) between the veins of the leaf blade or on the petiole. On the blade the water-soaked area gradually enlarges to form a lesion (Fig. 1, *B*, *b*) from 10 to 15 mm. in length, parallel to and between the veins. As the lesion increases in length the middle portion dries (Fig. 1, *B*, *b*) leaving a progressive water-soaked margin of 3 to 5 mm. at each end. Transverse growth of the lesion is very slight and it seldom crosses a vein (Fig. 1, *C*, *c*, *d*). On the petiole, lesions (Fig. 1, *A*) are roughly circular and usually retain that form. The fusion or numerous lesions may constitute one large diseased area (Fig. 1, *F*) covering most of the upper surface of the petiole.

The natural color[2] of the leaf of *Washingtonia filifera* varies from cedar-green to calla-green. An infected leaf may vary from the normal color to a yellowish oil-green or an oil-green. The oil-yellow-green of the young water-soaked spots does not conspicuously stand out against the normal green of the leaf. As the lesion increases in size the water-soaked part changes to dark-ivy-green and the drying internal portion becomes olive-buff to fire-clay colored surrounded with the dark-ivy-green, water-soaked tissue. Eventually, on complete drying out of the lesion, the tissue takes on an olive-buff to deep-olive-buff color. The changes that take place in the lesions on the petiole parallel those in lesions of the blade, although the final stage of infection may here present a somewhat darker shade.

Economically, the leaf-spot disease of Washington palm is important only to the extent to which the spots may detract from the ornamental appearance of the leaves. The infection appears incapable of killing an entire leaf. Since the most extensive infection is limited to the older leaves of the palm, the diseased parts may be mostly removed in seasonal pruning.

[1] The author gratefully acknowledges the assistance and criticisms of Dr. J. G. Brown, Professor of Plant Pathology, and of Mrs. Alice Boyle, Research Assistant, during the progress of the work. His thanks also are due to Dr. R. B. Streets, Associate Professor of Plant Pathology, who made the photomicrographs used in this paper.

[2] All colors mentioned in this paper refer to "Color Standards and Nomenclature" by Ridgway.

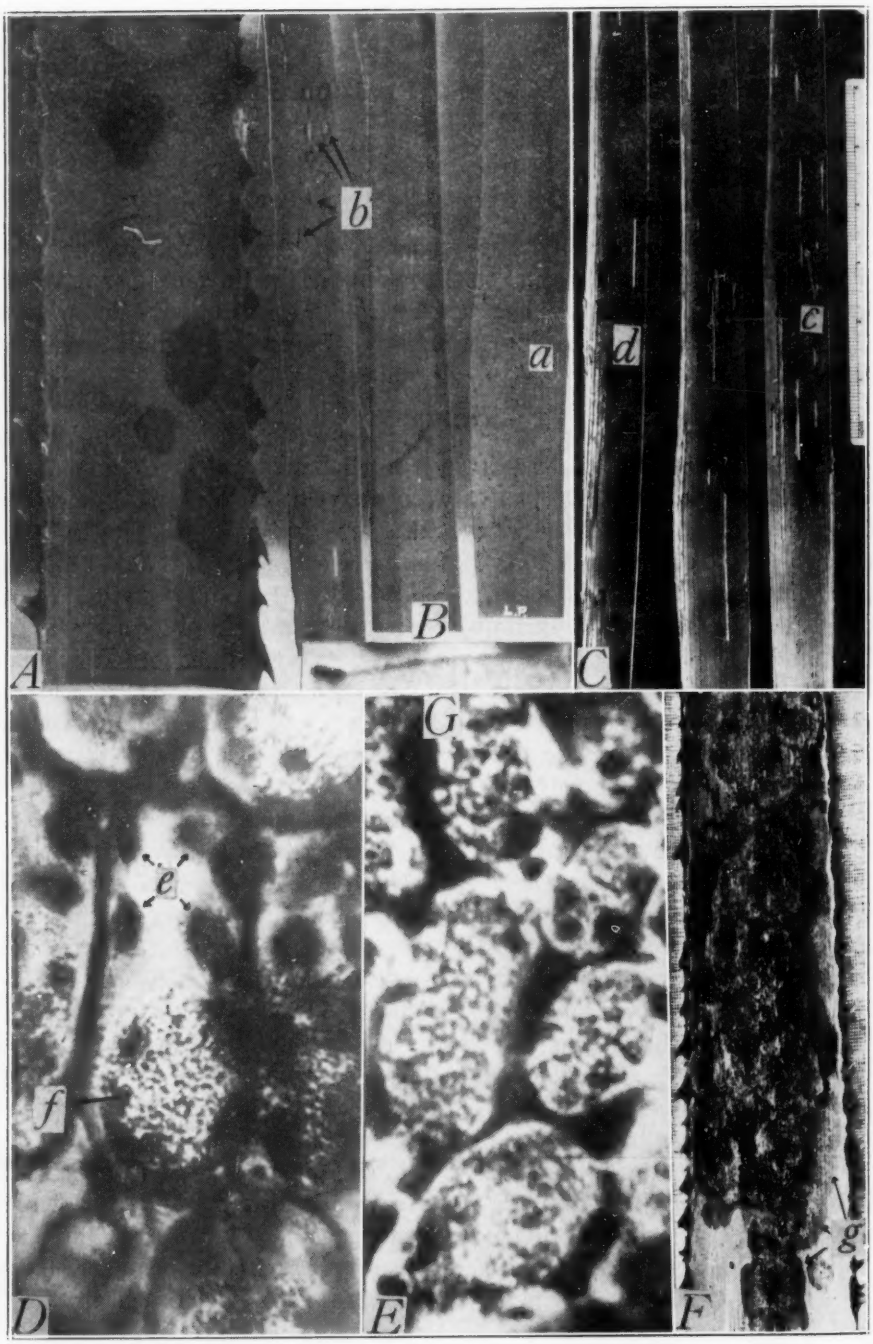

FIG. 1. *A*. Section of petiole with water-soaked lesions. *B*. Pieces of three lobes of leaf-blade showing progressive stages of lesions: *a*, numerous early stages of infection; *b*, lesions elongated between veins of leaf; the older ones with internally dry and necrotic tissues. *C*. Parts of lobes of blade with advanced linear lesions: *c*. fusion of lesions;

ISOLATION OF THE CAUSE

Small sections of tissues containing water-soaked areas were cut from infected leaves, dipped in mercuric chloride, 1:1000, for 15 to 30 seconds, and placed on P.D.A., pH 7.0. On the plates appeared 4 different types of colonies, of which 3 were, respectively, a Gram-positive bacillus, a smaller Gram-positive rod, and a yeast. The fourth type of colony, present in the majority of cases and that appeared in almost pure culture, was a small Gram-negative rod. Because the last showed greater probability of being the primary causal organism, it was selected for study. Poured plates of crystal-violet agar (1 cc. of 1:1000 crystal violet in aqueous solution per plate of P.D.A.) gave pure cultures of the bacterium.

The isolated organism in pure culture was inoculated into campus palms that appeared to be free from infection, by spraying the inoculum on the under side of the leaf blade and also by the needle method in both petiole and blade. Seven days later inoculations of both series had taken, small water-soaked lesions appearing at the base of the angles formed by the junction of the lobes with the petiole and around needle punctures. That these artificial infections did not progress so rapidly as the natural ones was attributed to the fact that the inoculations were made in late spring, a period in which little or no natural infection has been found. Re-isolations made from the lesions resulting from inoculations gave, with few exceptions, a pure colony of a bacterium identical in appearance with that used in the inoculum. Physiological tests of the bacterium gave results that corresponded precisely with those of the organism isolated from the natural lesions.

DESCRIPTION OF THE BACTERIUM

The cause of the leaf spot on *Washingtonia filifera* is a non-spore-forming, Gram-negative, motile, polar-flagellate (1–3 mostly uni-) rod (Fig. 1, *G*) with rounded ends, 1.61 $\mu \times 0.69\ \mu$, occurring singly or in chains of 3–4, forming entire-margined, smooth-surfaced, convex, glistening, white to cream-colored, butyrous, circular colonies 1½ to 2 mm. in diameter (P.D.A., 10 days, 20° C.) and filiform colonies on slants of the same medium. Physiologically, the bacterium is aerobic, has a T.D.P. of 47–48° C. (bouillon, 10 min.) is gelatine-liquefying, non-starch-hydrolysing, non-indol-producing, non-nitrate-reducing, and produces H_2S in very small amounts; in milk it is non-curdling, peptizing, and imparts a green color to litmus milk within 7 days. It gives an acid reaction to glucose, fructose, l-arabinose, in 24 hours; to galactose and xylose in 48 hours; no acid in sucrose, lactose, cellibiose, maltose, mannitol, d-sorbitol, glycerine, salicin, and raffinose in 6 days, and produces no gas within 6 days in any of the above carbon sources. For the bacterium is suggested the name, **Phytomonas washingtoniae.**

EFFECT ON HOST

The conditions that seem to be most suitable for infection of the host with the leaf-spot bacterium are those of periods with rain and low tempera-

d, an advanced stage of fusion. *D*. Photomicrograph of longitudinal section of a parenchyma cell from water-soaked area of leaf: *e*, normal chloroplasts in cytoplasm; *f*, bacteria that have invaded base of cell through broken wall. *E*. Disintegrating parenchyma cells from the drying area of lesion on leaf blade, in which masses of bacteria completely fill cells. *F*. Lesions that started as shown in *A* and *B* have coalesced and now practically cover the entire upper surface of a long petiole; *g*, water-soaked margin of lesion. *G*, Polar, uniflagellate bacterium.

ture. Therefore, the disease occurs in late fall and winter. Upon the appearance of drier and warmer weather, the causal bacterium becomes inactive and very little, if any, new infection follows. From the results of spraying with bacterial suspensions and the appearance of normally infected leaves, it is evident that the bacterium enters the host *via* the stomata that are present in both upper and lower epidermis of the leaves. Infection more commonly occurs on the ventral side. Natural infection is present mainly on the older, more horizontal leaves that hold liquid moisture longer than the younger vertical leaves.

Lesions will penetrate the entire thickness of the blade of palm leaves, but seem to affect only shallowly the surface of the fleshy petiole; they are limited to the parenchyma (Fig. 1, *D, f; E*) tissue. That lesions seldom cross a vein in the leaf is probably due to the small amount of parenchyma between vein and epidermis.

Attempted inoculations of green and semi-ripe date fruits gave no infection. Examination of the date palms on the campus showed various lesions on petioles and leaflets, but attempts at isolating the same causal organism occurring on *Washingtonia* were negative.

SUMMARY

A new disease has been reported for *Washingtonia filifera*. This disease, which affects the leaf of the palm, does not appear to be capable of killing the entire organ and is economically important only in so far as it alters the ornamental value of the leaves by spotting them. As yet the disease has not been observed on palms other than *Washingtonia*. The name of **Phytomonas washingtoniae** has been given to the causal organism.

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INHIBITION OF PHYMATOTRICHUM SCLEROTIA FORMATION BY SULPHUR AUTOCLAVED WITH SOIL

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(Accepted for publication May 18, 1943)

In addition to the well-known toxic effects of sulphur on fungi, small quantities of finely-ground sulphur, thoroughly mixed and autoclaved with soil, have been found to inhibit the formation of sclerotia by the cotton root-rot fungus (*Phymatotrichum omnivorum*). Mycelial growth of the fungus did not appear to be affected by the sulphur treatment, although no thickening of the mycelial strands to form the sclerotia took place when about 1 part (or more) of sulphur was added to 2000 parts of air-dry soil. This effect of sulphur is different from the prevention of infection by *P. omnivorum* reported by Taubenhaus and Ezekiel,[1] in which case an increase in soil acidity was caused by the oxidation of sulphur by soil organisms.

The same type of sterile-soil culture as was used[2] for obtaining sclerotia of *Phymatotrichum omnivorum* in quantity were employed in this study. Air-dry Houston black clay soil (about 8 per cent moisture content on an oven-dry basis) was passed through a 20-mesh sieve and definite amounts of dusting sulphur (98 per cent passing a 325-mesh screen) were added to weighed amounts of the soil. Different brands of dusting sulphur, with and without a conditioning agent, were used with like results. After thorough shaking in a stoppered flask, 100 g. aliquots of the sulphur-treated soil were placed in 250 ml. flasks. The same amount of soil with no sulphur added was placed in other flasks for controls. After adding definite amounts (25 to 40 ml.) of tap water to the soil, 5 g. of sorghum seed were placed on the surface of the soil and the flask plugged with cotton and sterilized at 15 lb. pressure for one hour. The flasks were inoculated with a pure culture of *P. omnivorum* on agar. The cultures were allowed to grow for 5 weeks at room temperature and the sclerotia were separated by washing the soil through a 40-mesh sieve. They were then placed on filter paper for about an hour before weighing.

Table 1 shows the average fresh weights (each figure represents an average of 10 flasks) of sclerotia obtained from two series of the variously treated soil cultures and from the checks. At each of the soil-moisture levels, no sclerotia were formed when sulphur was added at the rates of 1 part per 500 or 1000 parts of soil. Only traces of sclerotia were found at the next lower concentration of sulphur (1:2000). At the 1:4000 concentrations of sulphur, about one-half of the normal amount of sclerotia were produced and at 1:8000 the amount of sclerotia produced was practically equal to that in the nontreated soil. There was little difference in the effects of the

[1] Taubenhaus, J. J., and W. N. Ezekiel. Relation of soil acidity to cotton root rot. Tex. Agr. Exp. Stat. Bull. 545. 1937.

[2] Dunlap, A. A. A convenient soil-culture method for obtaining sclerotia of the cotton root rot fungus. Amer. Jour. Bot. 28: 945–947. 1941.

sulphur between the various levels of moisture. Figure 1 shows Petri-dish cultures of the fungus—one side of each Petri dish contained soil to which sulphur was added and the other side (showing sclerotia on the bottom) was filled with nontreated soil.

The seeds on the surface of the sulphur-treated soil were covered with mycelium and completely decayed by the fungus. Mycelial strands were also seen growing close together over all surfaces of the treated soil, and hyphae were found throughout the soil mass. This condition was found to exist when as much as 1 part of sulphur to 65 parts of soil were used. Other experiments made with navy beans, corn, cowpeas, and barley as

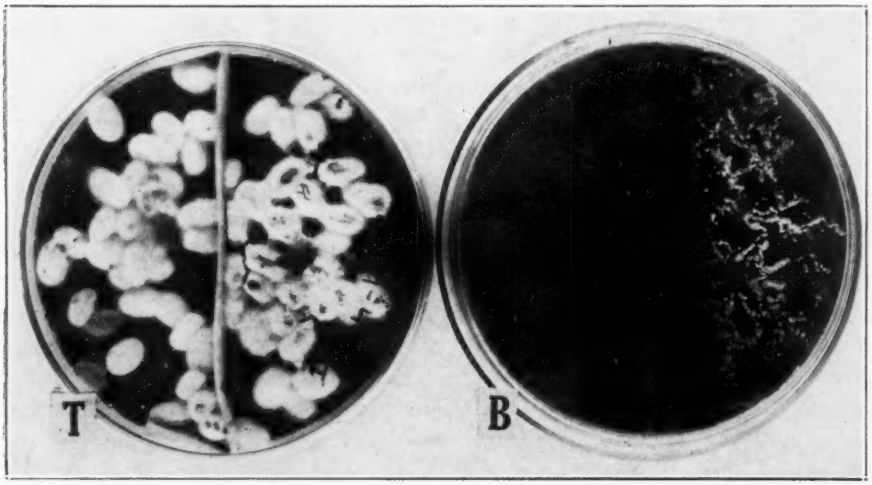

FIG. 1. Petri-dish cultures of *Phymatotrichum omnivorum* on Houston black clay soil with navy beans on the soil surface for nutrient, 20 days after inoculation. T, top of a culture; B, the bottom of a similar culture. The soil in the left half of each culture was treated before autoclaving with 325-mesh sulphur at 1: 1000; the soil in the right side (with sclerotia) was nontreated.

sources of nutrient gave results similar to those with sorghum seed. Also, no sclerotia were formed in a noncalcareous, slightly acid soil that was treated with similar quantities of sulphur and autoclaved. The addition of calcium carbonate to acid soils (5.4 to 6.1 pH) did not make the sulphur treatments with smaller amounts of sulphur any more effective.

As compared with sulphur treatments, sclerotia formation was not proportionally decreased by the addition of dry lime-sulphur (Sherwin-Williams) to the dry soil, in amounts supplying sulphur in excess of 1 : 1000. Likewise, sodium sulphide (Merck, reagent), sodium polysulphide (fused, 60 per cent), potassium sulphide (Merck, Tech.), and calcium sulphide (U.S.P.) did not prevent sclerotia formation when dissolved in the water that was added to successive layers of the air-dry soil in the culture flask before autoclaving. Treatment of the soil with materials containing organic mercury compounds (Semesan, Sanoseed) and with cuprous oxide (red

TABLE 1.—*Effect of sulphur on formation of sclerotia in autoclaved soil cultures of Phymatotrichum omnivorum with varying soil moisture*

Sulphur application	Fresh weight of sclerotia per flask				
	Amount of water per flask				Average
	25 ml.	30 ml.	35 ml.	40 ml.	
	g.	*g.*	*g.*	*g.*	*g.*
1: 500	0.0	0.0	0.0	0.0	0.0
1: 1000	0.0	0.0	0.0	0.0	0.0
1: 2000	0.3	0.3	0.2	0.1	0.2
1: 4000	1.2	2.0	3.0	2.7	2.2
1: 8000	3.9	4.6	4.0	3.9	4.1
None	4.0	4.6	4.1	3.9	4.2

Cuprocide), in amounts similar to the quantities of sulphur used, likewise failed to prevent sclerotia formation under these conditions.

It was apparently necessary that a finely divided sulphur be used for most effective inhibition of sclerotia formation and that the sulphur be thoroughly mixed and autoclaved with the soil. This inhibition was much less marked when coarse soil sulphur (16-mesh and finer) was used. This treatment would represent a smaller sulphur dosage because of the reduced amount of particle surface as compared with dusting sulphur. Placement of the fine sulphur on the surface of the soil without mixing in the flask did not inhibit sclerotia formation. Treatment of the soil with a solution of sulphur in carbon disulphide—allowing the carbon disulphide to evaporate before placing the soil in the flask—prevented sclerotia formation and this method required as much sulphur (for equal results) as mixing the dry dusting sulphur into the soil.

The autoclaving process may have resulted in the formation of a toxic sulphur material that inhibited the sclerotial stage of the fungus. Autoclaving appears necessary because sclerotia were formed in abundance in similar soil-sulphur mixtures that were sterilized in a hot-air oven at 180–200° C. for 5 hours, with the sterile water and seeds added later. No significant differences in pH were found in the Houston black clay soil, following autoclaving with the small amounts of sulphur. A toxic compound, however, apparently was not formed from the sulphur compounds added to the soil before autoclaving. There was a distinct odor of hydrogen sulphide in the soil cultures following growth of fungus, in cases where enough sulphur was added to prevent the formation of sclerotia. In flasks sealed with Parafilm after inoculation, a reduction of aerial growth of mycelium was noted in 1:1000 sulphur-treated soil although abundant aerial growth was present in the 1:4000 flasks. (Few sclerotia are formed in sealed flasks.[2]) The hydrogen sulphide was probably formed from reduction of the sulphur by the fungus, as similar non-inoculated flasks showed no odor after 5 weeks. Wettable and colloidal sulphurs were found to

[2] Dunlap, A. A. *l.c.*

lessen the rate of growth of *Phymatotrichum omnivorum* in both liquid and agar media. In liquid cultures, small amounts of potassium or calcium sulphide prevented growth of the fungus entirely.

SUMMARY

When thoroughly mixed with the soil and autoclaved, one part of 325-mesh dusting sulphur to 1000 parts of air-dry black Houston clay soil resulted in complete inhibition of sclerotia formation by *Phymatotrichum omnivorum*, with no apparent effect on mycelial growth. Only traces of sclerotia were formed when a sulphur : soil mixture of 1 : 2000 was used and no inhibitive effects of the sulphur were noted at 1 : 8000. It was necessary to use a finely-ground sulphur and to mix the sulphur thoroughly with the soil in order to prevent sclerotia formation. The inhibitive effect was due possibly to the formation of a toxic compound upon autoclaving.

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ASSOCIATION BETWEEN NEMATOSPORA PHASEOLI AND THE GREEN STINKBUG[1]

J. G. Leach and Genevieve Clulo

(Accepted for publication May 20, 1943)

In 1940 Leach (2) reviewed the literature dealing with stigmatomycosis and its transmission by various species of plant bugs. Available published information led him to make the following statement. "The universal association of species of *Nematospora* with the insects in such widespread regions of the world would suggest that the association is more than a casual one. A thorough study of the possible symbiotic relationships between insect and fungus should be made. It is significant that no satisfactory explanation of the method of survival of the fungi over winter has been offered by any worker on the disease. The question of whether the fungus is constantly associated with the insects throughout their life history or whether they pick it up from some unknown source has never been answered."

One of the most common forms of stigmatomycosis in the United States is yeast spot of beans, caused by *Nematospora phaseoli* Wingard. Yeast spot has been studied extensively by Wingard (5) who first presented experimental proof of its transmission by the stinkbug. In regard to the method of transmission, Wingard (6) stated "These results indicate that *Nematospora* is carried by the above-named insects, but whether it is carried internally or externally remains to be determined. Nowell has attempted to isolate it from *Dysdercus delauneyi* and *Nezara viridula* but without success, and I have had the same experience with *Nezara hilaris*. Other yeasts and yeast-like fungi are very readily obtained from this insect but I have consistently failed to isolate *Nematospora phaseoli*. I am inclined, nevertheless, to believe that these insects are themselves infected with the organism. The negative results obtained thus far are very probably due to improper technique."

Stigmatomycosis appears to occur most abundantly in the tropical and subtropical regions of the world, although some forms of the disease occur in the warmer regions of the temperate zones. Yeast spot of Lima beans is common in southeastern Virginia, but has been observed only a few times in West Virginia. In the summer of 1941 a study of the association of yeast spot and the green stinkbug was undertaken with the hope of throwing some additional light on the method of transmission and possible overwintering of the pathogen within the body of the insect vector.

Although a fairly extensive survey of several localities in West Virginia was made, the disease was not found. The stinkbug (*Nezara hilaris* Say), however, was fairly prevalent on Lima beans and various noncultivated hosts. A careful search disclosed none of the disease, even when the insects were very numerous on Lima bean plants. Isolations were made from the

[1] Published with the approval of the Director, West Virginia Agricultural Experiment Station, as Scientific Paper No. 307.

feeding punctures of the bug on Lima bean pods, as well as from both internal and external organs of the stinkbugs, both nymphs and adults, but these failed to yield a culture of *Nematospora*. Several kinds of culture media were used on all of which the yeast grows well. For comparison with these specimens some stinkbugs from southeastern Virginia collected from Lima bean plants affected with yeast spot were obtained through the courtesy of Dr. S. A. Wingard. *Nematospora* was isolated readily from the surface of these insects but none was obtained from the internal organs aseptically dissected from surface-sterilized insects.

As a further check on possible internal transmission, specimens of bugs, both nymphs and adults, were imbedded in paraffin, sectioned and stained with the modified Gram-Weigert method (6). A careful search did not reveal *Nematospora* within the body of the specimens from either source. In certain individual insects microorganisms were present in the gastric caeca, but these obviously were not *Nematospora*. They were in all probability the microsymbiotes described by Glasgow (1). Efforts to grow these in artificial culture were unsuccessful. Although they were removed aseptically from freshly dissected insects and plated in agar, they did not grow.

The possibility of internal biological transmission seems unlikely when one considers the relative diameters of the yeast cells and the food channel of the insect's mouth parts. Measurements of the food channel of adult insects show that it rarely exceeds 12 μ in diameter, while the salivary channel is several μ smaller. Although some of the immature vegetative cells of *Nematospora* may be smaller, the great majority of mature cells are from 10 to 20 μ in diameter, much too large to permit ready passage.

The absence of yeast spot in association with stinkbugs on Lima beans in West Virginia indicates that the association of the disease with the stinkbugs is not a constant one, even though the disease may depend upon the insect for inoculation. The failure to find *Nematospora* within the body of insects taken from plants affected with yeast spot would indicate that transmission may be entirely external and mechanical.

Nowell (3), in his study of the internal disease of cotton bolls in the West Indies, reported 4 species of fungi transmitted by several species of bugs. All 4 of these fungi were found affecting the fruits of several species of plants, mostly legumes, that serve as hosts for the insect vectors before they migrate to the cotton bolls. The inference from Nowell's work seems to be that these weed hosts serve as sources of contamination of the bugs. Although Nowell reported no histological work with the bugs, he showed in one experiment that bugs reared on healthy plants did not transmit the fungi when allowed to feed on cotton bolls. Bugs from diseased plants transmitted the fungi readily.

Even though *Nematospora phaseoli* does not survive within the body of hibernating adult bugs, it could possibly overwinter as resistant cells or spores on the external parts of the insect. It seems more likely, however, that the bugs become contaminated in the spring by feeding on infected

material. Underhill (4) reports that Lima beans are not the preferred host of the stinkbug and that they usually migrate to beans after feeding for some time on certain preferred noncultivated plants. This possibility is supported by the greater prevalence of yeast spot in the regions of mild winters, where infected plant tissues would not be destroyed by freezing and where actively growing yeast cultures would more likely survive and be available. If the pathogen survives the winter in this way the discovery and elimination of the source of contamination might offer a practical means of control. Very little is known of the host range of *Nematospora phaseoli* in nature. This should be determined, but it can be done effectively only in milder regions where the disease is more abundant than it is in West Virginia.

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PHYTOPATHOLOGICAL NOTES

Occurrence of the Imperfect Stage of Sclerotinia laxa on Prunus cerasus in Wisconsin.—On June 18, 1941, trees of the Montmorency and Early Richmond varieties of *Prunus cerasus* L. in a commercial orchard near Bailey's Harbor, Door County, Wis., were observed to be severely affected by a blossom and twig blight (Fig. 1, A). No sporulation of any known pathogen

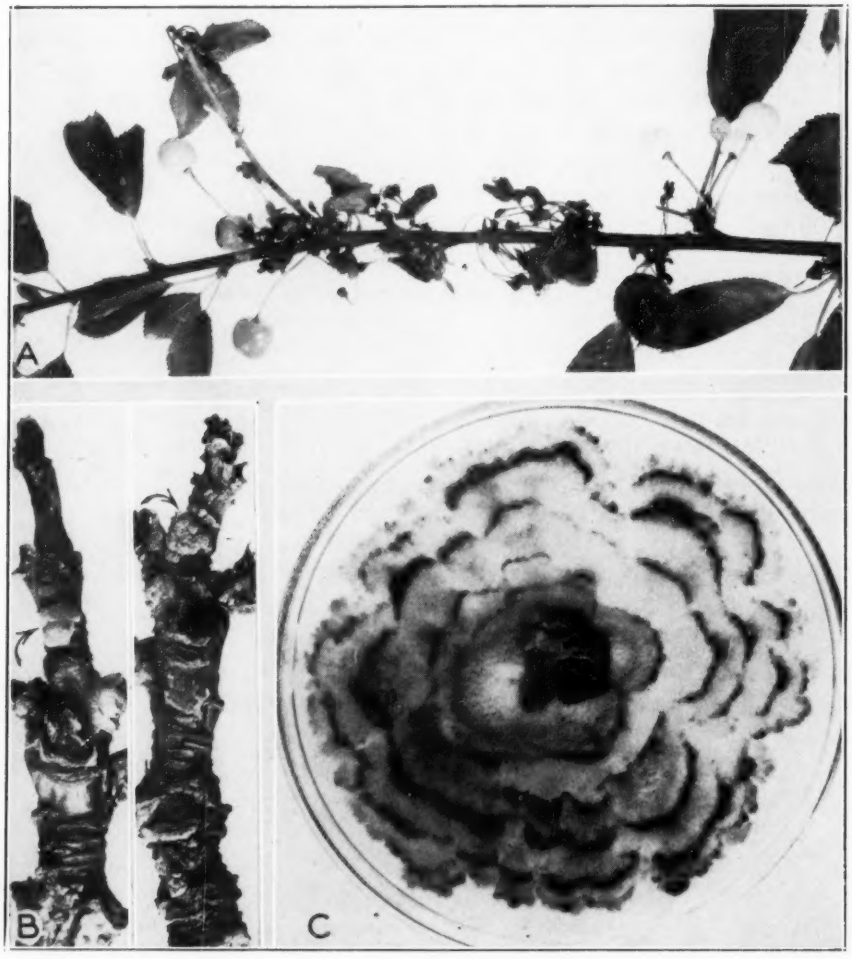

FIG. 1. A. Blossom and twig blight of Early Richmond cherry, incited in the current season by *Sclerotinia laxa*. B. Early Richmond fruit spurs blighted in the preceding year, showing sporodochia (at arrows) of *S. laxa* developed in the current spring. C. *S. laxa* on potato dextrose agar after three weeks at 24° C.

was observed on the diseased parts, but plate cultures from internal tissues of diseased twigs consistently yielded a fungus showing the cultural characters of *Sclerotinia laxa* Ader. and Ruhl.[1,2,3]

[1] Ezekiel, Walter N. Fruit-rotting Sclerotinias. II. The American brown-rot fungi. Md. Agr. Exp. Stat. Bull. 271: 87–142. 1924.

When the same trees were examined in the spring of 1942 abundant sporodochia of the fungus were found. These occurred most commonly on the blighted fruit spurs of the preceding year (Fig. 1, B), but also on the remains of petioles and midribs of the leaves and persistent parts of the overwintered blighted blossoms. A severe local outbreak of this blossom and twig blight occurred in this orchard in the spring of 1942, but no sporodochia were observed on lesions developed in the current season. However, in the spring of 1943 a few sporodochia were observed on blossoms blighted in the current year. Platings from such sporodochia yielded *Sclerotinia laxa.*

The fungus was isolated at will (Fig. 1, C) from internal tissues of blighted twigs in spring and fall. It was grown on potato dextrose agar in comparison with *Sclerotinia fructicola* (Wint.) Rehm. It was used in the greenhouse to inoculate blossoms and twigs of *Prunus cerasus* (Montmorency), on which it incited symptoms characteristic of *S. laxa.* Control plants were not diseased. The organism was reisolated from an infected plant and found to have the same cultural characters as the cultures used for the inoculations. Reinoculation from these cultures resulted in typical infection.

The Door County cherry district has not been completely surveyed for *Sclerotinia laxa,* and it is not yet possible to evaluate the danger of this pathogen to the Wisconsin cherry industry. Local outbreaks similar to the one reported have been observed in certain nearby orchards, and the fungus has been found in less conspicuous development in several other locations in the cherry district of Door County. Experiments with both eradicant and protectant sprays for the control of this brown-rot disease have been initiated.

Sclerotinia laxa, the common brown-rot fungus of stone fruits in Europe, has long been known to occur in Europe and the British Isles, Manchuria and Japan, and along the Pacific Coast of North America.[3] So far as the writers are informed, its occurrence in central or eastern North America has not hitherto been proved.[1,2,3]—G. W. KEITT, J. DUAIN MOORE, E. C. CALAVAN, and J. R. SHAY, University of Wisconsin, Madison, Wisconsin.

Masking of Leaf Symptoms of Sour-cherry Yellows by Temperature Effects.—The leaf symptoms of cherry yellows,[1,2,3,4] a virus disease, may

[2] Ezekiel, Walter N. Presence of the European brown-rot fungus in America. Phytopath. **15**: 535–542. 1925.

[3] Harrison, T. H. Brown rot of fruits and associated diseases of deciduous fruit trees. I. Historical review, and critical remarks concerning taxonomy and nomenclature of the causal organisms. Jour. and Proc. Roy. Acad. N. S. Wales **67**: 132–177. 1933.

[1] Keitt, G. W., and C. N. Clayton. A destructive bud-transmissible disease of sour cherry in Wisconsin. Phytopath. **29**: 821–822. 1939.

[2] Rasmussen, E. J., and Donald Cation. A progress report on the yellow-leaf disease of sour cherry. Mich. State Hort. Soc. Ann. Rept. **71**: 100–105. 1942.

[3] Hildebrand, E. M., G. H. Berkeley, and D. Cation. Handbook of virus diseases of stone fruits in North America. Mich. Agr. Exp. Stat. Misc. Pub., 76 pp., 1942.

[4] Keitt, G. W., and C. N. Clayton. A destructive virus disease of sour cherry. Phytopath. **33**: 449–468. 1943.

vary greatly in degree of expression in different seasons or regions. The cause of these variations, which have been a substantial barrier to the progress of investigations of this disease, has not been explained. The present paper gives a preliminary report on experiments indicating that temperature is a sharply limiting factor in the expression of leaf symptoms of this disease.

In a preliminary experiment in the spring of 1942, potted Montmorency trees in which buds from Montmorency trees affected by yellows had been inserted were incubated comparatively in greenhouses in which the temperatures were approximately 16, 20, 24, and 28° C., respectively. Some of these trees were budded in the greenhouse in January; others had been brought in a dormant condition from the field, where they had been budded and had shown yellows symptoms. The leaf symptoms of yellows developed freely in the 16° house. Only one tree in the 20° house showed any symptoms of the disease, and no symptoms appeared at 24 or 28°. All of the trees of this experiment were placed out of doors during the summer and early winter of 1942 and returned in December to the same greenhouses they had occupied in the spring. Those in the 16° house showed typical leaf symptoms of yellows by March 17, 1943, whereas none of those at the higher temperatures showed symptoms of the disease during the 1943 greenhouse season.

In another experiment in the spring of 1942, potted Montmorency trees in the greenhouse were budded from yellows sources, carried through the summer and early winter out of doors, and then brought into the greenhouses for tests on the expression of leaf symptoms. Four such trees were placed at each of the following temperatures: 16, 20, 24, and 28° C. Typical leaf symptoms of yellows developed freely on all 4 trees in the 16° house in the spring of 1943. No symptoms appeared on the trees incubated at the higher temperatures.

On January 18, 1943, dormant buds from a Montmorency tree affected by yellows were inserted in dormant Montmorency nursery trees that had recently been potted in the greenhouse. Three of these budded trees were incubated at each of the following greenhouse temperatures: 16, 20, 24, and 28° C. On March 17, two of the trees at 16°, and none of the others, showed leaf symptoms of yellows. None of the other trees developed symptoms of yellows during the greenhouse season of 1943.

Because of the indications in the preliminary experiment of 1942 that 16° C. is a favorable temperature for development of the leaf symptoms of yellows, a greenhouse in which most of the experimental potted trees were carried in 1943 was held at comparatively low temperature. At night the temperature varied from 12 to 16°, but in the middle of the day it often ran in the range of 24 to 28° C. On scores of sour cherry trees incubated in this greenhouse, the typical leaf symptoms of yellows developed freely.

The results thus far available indicate clearly that 16° C. is a favorable temperature for expression of the leaf symptoms of cherry yellows and that

these symptoms are masked at approximately constant temperatures of about 20° C. or higher. Closer studies of the effects of diurnal fluctuations in temperature and correlations with epidemiological data remain to be made. In the meanwhile, the use of potted plants at suitably controlled temperatures in the greenhouse affords favorable opportunity for experimentation on many aspects of cherry yellows and on other virus diseases with which it may be associated.—G. W. KEITT and J. DUAIN MOORE, University of Wisconsin, Madison, Wisconsin.

A New Mosaic Disease of Beans.—In 1939, Michelite pea beans in two fields near Batavia, New York, were observed, infected with a mosaic virus producing symptoms that, to all appearances, were identical with those of the common bean mosaic. The incidence of the disease was so high that the two fields were not harvested. This was the first time, as far as the writers are aware, that the Michelite had been grown in New York State, and the fact that it was susceptible to the mosaic virus was surprising, since the variety[1] had been reported to be resistant. Also, during the last few years many complaints have been received from growers stating that the Robust, the outstanding pea bean for 25 years, was beginning to exhibit large percentages of plants showing mosaic symptoms. The Robust bean, shortly after its introduction, had been proved immune from the virus[2] and had remained so for years. Whether or not the occurrence of this disease in the Robust was due to impurities in the stock could not be determined from observation.

In 1942 a number of samples of both Robust and Michelite beans were obtained from as reliable sources as possible. Tests with an isolate of mosaic virus, obtained from a plant grown in New York State, demonstrated that these two varieties were 100 per cent susceptible to the virus isolate used. Later, 6 isolates of mosaic virus taken from diseased seedlings, 3 of which the seed had been grown in New York State, 1 in California, 1 in Idaho and 1 in the Canal Zone, were used in inoculation experiments. These isolates were maintained on Red Kidney beans that previously had been shown free from mosaic. The Robust and Michelite proved susceptible to 2 isolates of the virus from New York seed, but immune from the other 4 virus isolates.

These results clearly demonstrate that there are 2 strains of the bean-mosaic virus or, at least, that there are two virus entities producing a mosaic disease on this plant. The symptoms produced by both are essentially those described for the common bean mosaic, and both viruses are seed-transmitted. Further experiments proved that the Red Kidney and the Bountiful are susceptible to the 2 viruses; that the Norida, Red Mexican 3 (Univ. Idaho), and Great Northern 15 (Univ. Idaho), together with the Robust and Michelite, are susceptible to what might be called the new strain of the

[1] Down, E. E., and J. W. Thayer. The Michelite bean. Mich. Agr. Exp. Stat. Sp. Bull. 295. 23 pp. 1938.

[2] Reddick, Donald, and Vern B. Stewart. Varieties of beans susceptible to mosaic. Phytopath. 8: 530–534. 1918.

virus, but not to the old; and that the Great Northern 1[3] and 59 (Univ. Idaho), Ashley's wax (U.S.D.A.), Cooper's wax (U.S.D.A.), and Refugee 5 (U.S.D.A.) are immune from both strains. The fact that in certain bean varieties there exists immunity from the virus that is infecting the pea beans is hopeful. Breeding work is underway to combine immunity from the 2 viruses in a small white type of bean of desirable characters.—B. LORIN RICHARDS and WALTER H. BURKHOLDER, Department of Plant Pathology, Cornell University, Ithaca, New York.

A Foliar Mottle and Necrosis in Chippewa Potatoes Associated with Infection by a Strain of the Potato X Virus.—The rather obscure tuber-perpetuated mosaic disease described in this note could not be definitely identified with any known potato virus. It was first noticed in a few tuber units of Wisconsin-grown Chippewa potatoes in a southern test planting in 1939 and again in 1941. In 1942 and 1943 additional Chippewa specimens were obtained from a large seed planting in northern Wisconsin and in an early table-stock planting of the same seed in the central area. Although the symptoms in the field bear some resemblance to early symptoms of mild mosaic on varieties such as Cobbler and Triumph, they are easily distinguished from it by characteristic irregular chlorotic mottle patches in the intercostal areas of the upper leaves of young plants and by the small scattered necrotic flecks on the older leaves. Later the irregular flecking is manifest to a slight extent in the upper leaves. Foliage symptoms vary in severity under both field and greenhouse conditions but are within a well-defined pattern range, and infected plants can be easily recognized. Lethal acronecrosis has not been observed under any conditions. External and internal stem and petiole tissues of affected plants are normal, and cells of the xylem, phloem, and cortex show no discoloration. Tubers are slightly smaller than normal but show no manifestations of infection.

Repeated aphid (*Myzus persicae* Sulz. and *Macrosiphum solanifolii* Ash.) transmission tests yielded negative results. When the virus was transmitted mechanically to Chippewa, Sebago, Triumph, Red Warba, and Russet Burbank varieties and to first generation potato seedlings derived from seed of Katahdin by selfing, the first sign of infection appearing on the inoculated leaves in 12 to 15 days as small, irregular, scattered, necrotic lesions, which penetrated the thickness of the leaf. The early recognizable systemic symptoms appeared as a slight, interveinal, chlorotic mottle of the younger leaves followed by necrotic flecking of the interveinal areas, which remained as isolated areas, showing no tendency to coalesce. Seedling 41956 is immune.

Artificially inoculated *Nicotiana tabacum* L. (var. Connecticut Havana No. 38) developed especially clear, small, light-colored, concentric ring-type lesions on the surface of inoculated leaves followed by systemic symptoms consisting of discrete ring-type and irregular line- and ring-patterns. There

[3] This variety was received from R. A. Emerson. The writers do not know whether it is of Idaho or U.S.D.A. origin.

is no evidence of vein chlorosis, vein-banding or mottle in the systemically affected tobacco plants. The ring-spot type of lesion is displayed to best advantage on *N. rustica*. Tobacco plants, previously infected with a mottle strain of potato X virus, did not show the above symptoms when reinoculated with the virus from diseased Chippewa plants, indicating strongly that the Chippewa virus is a variant of virus X. It would appear, therefore, that this variety, which is highly resistant to mild mosaic and is a masked carrier of common strains of potato X, is subject to infection by an uncommon strain of the latter, which causes mosaic mottle not unlike that of mild mosaic on Cobbler and Triumph. In preliminary temperature studies, symptoms developed in affected Chippewa plants more rapidly and severely at 16° to 20° than at 22° to 24° C., while they were entirely suppressed at 28° C.—R. H. LARSON, Wisconsin Agricultural Experiment Station, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

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Page 2, line 11, *read* r. p. s. *for* r. p. m.
Page 204, line 5, *read* (Fig. 3, D) *for* (Fig. 4, D)
Page 220, line 6 from bottom of page, *read Cercospora for Cerospora*
Page 225, line 1, *read Gloeocercospora for Gloeocerspora*
Page 308, line 6, *read* seeding *for* seedling
Page 328, line 12, *read* 50 cc. *for* 500 cc.
Page 430. In date line beneath title, *read* July 29 *for* July 28
Page 552, line of continuation of footnote 4 of preceding page *read* dark green *for* green dark
Page 567, literature citation 2 *read* Dalldorf *for* Daldorf
Page 624, delete footnote [2] from title
Page 628, lines 25 and 49, *read* McCallan *for* McAllan
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